

A Study of the Rectal Breathing-Apparatus in the Larvæ of Anisopterid Dragonflies. By R. J. TILLYARD, M.A. (Cantab.), B.Sc. (Sydney), F.L.S., F.E.S.

(PLATES 18-22, and 21 Text-figures.)

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INTRODUCTION.

THE present paper is the outcome of a suggestion made to me some three years ago by my friend Dr. F. Ris, of Rheinau, Switzerland. Being unable to spare the time to carry out the researches which he had begun, and of which he later on published a short account (23), he very kindly suggested that I should take up the subject, which promised to yield many interesting facts new to science. Through his generosity in giving me full information as to his methods, and in sending me a set of prints of his very beautiful but unpublished microphotographs, I was able to apply his methods to the rich Australian material at my disposal. The immediate result of this was the discovery of a number of new and interesting forms of rectal breathing-apparatus. Later, I undertook the study of the finer details and histology of these organs by means of microtome-sections. This method again yielded a number of new facts not less in interest than the former, although the great

complexity of the gill-formation made the investigation in almost every case not only a difficult one, but very long and tedious.

In order to avoid prolonging the investigation unduly, I have found it necessary to postpone not only the physiological study of the respiration, as carried on in the rectum, but also to confine my attention almost entirely to mature or fairly mature nymphs. A complete phylogenetic study demands, of course, a thorough investigation also into the successive ontogenetic changes that take place at each ecdysis throughout the growth of each larva studied. This would, however, be a matter not of months merely, but of years. In the twelve months during which this work has been carried out, I was only able to study the ontogenetic gill-development in two genera (*Aeschna* and *Anax*)—fortunately, genera which throw much light on the phylogenetic problem. The object of the paper may therefore be briefly stated as an attempt at a complete morphological study of all obtainable forms of rectal breathing-apparatus in Australian Anisopterid dragonfly nymphs, with some phylogenetic deductions which appear to me to be fully warranted by the facts therein established.

Some of the facts made known for the first time in this paper do undoubtedly throw light upon the very difficult physiological questions involved. It seems to me, however, to be unsafe to draw physiological conclusions, as Sadones (25) has done, from purely morphological and histological studies. I therefore prefer to hold over this aspect of the question until a later date, when perhaps physiological experiments may have yielded more conclusive evidence than can be to-day offered in support of any theory of respiration in these insects. If at any point I have touched upon the physiological aspect of the problem, it is because that aspect happens to stand out very clearly in relation to the facts in hand, and not because they offer anything like a complete or satisfactory solution of the problem.

I should like to express my thanks to Professor W. A. Haswell, F.R.S., under whom this research has been carried out, for much kindly encouragement and sound advice; while to Dr. F. Ris I am indebted for the origination of the subject and also for valuable criticism during the progress of the research.

HISTORICAL SUMMARY.

In the appended Bibliography there will be found a complete list of all the authors known to me who have mentioned the rectal breathing-apparatus of Odonate larvæ. These peculiar rectal structures seem to have been noticed first by Swammerdam (28), though the actual recognition of them as a breathing-organ is probably due to Poupert (20). The respiratory function of the rectum was also known to Réaumur (22), de Geer (9), Cuvier (3), Duvernoy (6), Marcel de Serres (14), Lyonnet (13), Suckow (27), and

Ratzeburg (21). But it was Dufour (5) who made the first real advance in the knowledge of the true nature of these organs. He showed that tracheæ penetrated into the projecting folds of the rectum, which thus are actually of the nature of *tracheal gills*. To Dufour also we owe the first definite assertion that the complicated rectal breathing-apparatus studied by him in the larvæ of *Libellula* and *Æschna* did not occur in the Zygoptera. His discovery of three longitudinal folds in the rectum of *Calopteryx*, which he considered to be poorly developed breathing-organs, does not concern us directly here. But, as this function has been denied to them by recent authors, we may well point out that this aspect of the problem needs fuller investigation.

After Dufour, the subject was briefly touched upon by Milne-Edwards (17) and Leydig (12). The latter author was the first to discover the important fact that the finest tracheal capillaries ramifying in the gills do not end blindly in them, but form *complete loops*. Leydig, however, was not at all seized with the importance of this discovery, which he announced in two lines of text, without giving a figure. It is, therefore, rather to Oustalet (18) that the real honour belongs of investigating this point. His description and figures are far in advance of any previous work on the subject, and mark a very distinct step forward in the progress of our knowledge.

Following Oustalet, Chun (2) was the first to study the *histology* of the rectum. He gives a figure purporting to show a transverse section of one of the gill-lamellæ of *Libellula depressa*, Linn. But, as Sadones (25) has shown, this figure is incorrect in very many details; and, besides failing to recognize the existence of the tracheal loops, established by Oustalet, it certainly does not belong to the genus to which it is assigned by its author. In fact, it may be stated definitely that no histological structure belonging to any known Odonate gill-type can be found which at all corresponds with Chun's figure. It is, indeed, very difficult to explain how his figure originated, except as the product of an imagination rather more fertile than accurate.

After Chun, Poletaiëw (19) contributed a paper, which is of value in being the first evidence of a disagreement with the generic determination of one of the larvæ studied by Dufour and Oustalet. This larva, which we now know for certain to have belonged to the genus *Anax*, was called *Æschna* by these authors. Poletaiëw, in examining undoubted larvæ of the genus *Æschna*, failed to find the papillæ so carefully described by Oustalet. Hence, she questions the accuracy of the descriptions of that author and of Dufour. None of these authors seem to have attempted to breed out any of the larvæ which they were studying. The facts are, of course, well known to all Odonatologists at the present time, thanks to Ris (23), viz. that the gills of *Anax* bear papillæ, while those of *Æschna* do not.

Amans (1), Roster (24), Faussek (8), R. Martin (15), and Dewitz (4), have all contributed to the study of the structure of the rectum and its tracheal

connections, but without making any notable addition to our knowledge of these organs. Miall (16), East (7), and Latter (11) have contributed popular accounts of the same structures.

The greatest advance in our knowledge of the subject is undoubtedly due to Sadones (25), who, working under Professor Gilson (10), produced in 1896 a classical memoir on the whole digestive tract of the larva of *Libellula depressa*. The outstanding merit of this work is the careful and accurate study of the histology of the rectal epithelium, and the part played by it in the formation of the gill-lamellæ. Sadones's original contributions to the subject include (1) the discovery of the basal pads of the lamellæ; (2) the discovery of the small tubercles on the lamellæ, which prevent them from lying too closely upon one another; (3) the demonstration of the complete continuity of the rectal epithelium as a fine *syncytial matrix* beneath the whole branchial cuticle; and (4) the fixing of the definite position of the tracheal capillary loops as running in this syncytium. To him, also, we owe a very clear criticism of Chun's description and figure. Sadones, however, seems to us to have forsaken firm ground when he turns to the physiological aspect of the subject. His theories concerning the absorption of oxygen and the elimination of carbon dioxide, whether they be ultimately proved correct or not, show a distinct lack of understanding of the closed tracheal system as it exists in Odonate nymphs, and are quite unsupported by any of that definite evidence which the importance of the subject demands.

After Sadones, the subject remained untouched for sixteen years (except for a short account by Scott (26), who added nothing new to our knowledge) until Ris (23) published, in a short but very valuable paper, the results of his comparative studies on the rectal gills of a number of European Anisopterid larvæ. Ris described the gill-structures in the following genera:—*Cordulegaster*, *Gomphus*, *Onychogomphus*, *Brachytron*, *Æschna*, *Anax*, *Cordulia*, *Orthetrum*, *Libellula*, and *Sympetrum*. In these ten genera he recognizes six distinct types of gill-formation. He also shows how these structures are found to be both more morphologically complex and physiologically perfect as we pass from genus to genus along the phylogenetic tree, as it has been already constructed for us on the venational characters of the imagines. Ris made a very beautiful series of microphotographs of the various types of gills; but these were unfortunately never published, owing to the difficulty of finding a process which would reproduce them satisfactorily.

Following on all these authors, it now remains for me to extend the methods and observations of Ris to our rich Australian fauna, and also to attempt the more difficult histological study of each new type, on the lines adopted by Sadones in his classical memoir on *Libellula depressa*. These two objects achieved, there remain some interesting phylogenetic considerations to be given, which will be found included in this paper.

TERMINOLOGY.

Various names have been applied by the authors cited above to the rectal breathing-apparatus and its parts. As there seems to be little unanimity between them, and as definite English equivalents seem still to be lacking for the names given by the French and German authors to whom the progress of our knowledge is almost wholly due, I offer here the following terminology, which will be used throughout this paper:—

The *Rectum* proper consists of all that portion of the hind-gut lying posterior to the dilated ampulla (*pre-rectal ampulla*) into the anterior end of which the small intestine enters. It consists of two distinct portions, a small posterior or *anal portion* containing six longitudinal raised epithelial pads (the so-called “rectal glands”), and a much larger anterior or *respiratory portion* containing the breathing-organs. To this latter portion, with which this paper has to deal, the term *Branchial Basket* or *Gill-basket* will be applied.

The folds of the rectal wall, which project into the cavity of the rectum and perform the respiratory function, will be spoken of generally as the *gills* or *gill-folds*. In a gill-fold, each of the two walls forming the eversion will be called a *gill-wall*. The two gill-walls forming any given fold are therefore continuous with one another at the free distal border or edge of the gill-fold. Each gill-wall is formed of a thin *epithelial matrix* with an outer secreted *cuticle* of excessive fineness.

The thickened epithelium which occurs at the bases of the gill-folds will be spoken of as the *basal pad* (“bourrelet épithélial” of Sadones).

The peculiar mass of tissue which lies at the base of each gill, carrying the efferent tracheæ, and covered either on one or both sides by the basal pad, will be called the *hypobranchial tissue* (“tissu adipeux” of Sadones).

When the gill-basket of an Anisopterous larva is opened, the gills are seen to lie in six longitudinal rows at equal intervals apart. Each of these rows will be termed a *holobranch*. Two main types of gill-basket are readily recognizable. In the simpler type, each holobranch consists of a single *longitudinal gill-fold*, supported at regular intervals, to right and left alternately, by smaller *cross-folds*. This type of structure will be called a *Simplex System*. In the second and more complicated type, there are no continuous longitudinal gill-folds; but each holobranch consists of a double row of smaller gills arranged more or less transversely to the long axis of the gill-basket. Each of these rows will be called a *hemibranch*. The number of gills in a hemibranch varies from twelve to thirty. This second type of gill-structure will be called a *Duplex System*.

The exact positions of the gill-rows or gill-folds in a transverse section of the rectum are of the greatest importance, if one would correctly understand the homologies of these organs. Hence I propose to use what I may call the

Clock-Face Notation to indicate these positions. As there are always *six* holobranchs arranged at equal intervals from one another, it will be clearly seen that, in a transverse section, the middle lines or axes of symmetry of the holobranchs must lie at the six angles of a regular hexagon inscribed in the circle which represents the transverse section of the branchial basket. Now, if we take the numerals 1 to 12 and place them in their ordinary positions on the clock-face, the mid-dorsal position will be represented by 12, the mid-ventral by 6, and the other numbers will lie at intervals of 30° along the circumference. In the *Simplex System* the positions of the six holobranchs will then be found to correspond with the numbers 2, 4, 6, 8, 10, 12. But in the *Duplex System* we shall find that the middle line, or axis of symmetry, of each holobranch (which, in this case, is actually the line lying midway between each pair of hemibranchs) lies in one of the positions 1, 3, 5, 7, 9, 11.

Now it happens that in all Odonate nymphs the six so-called "rectal glands" of the anal portion of the rectum lie in the positions 2, 4, 6, 8, 10, 12. Sadones, who only examined the gills of the larva of *Libellula depressa*, which belong to the *Duplex System*, noticed that their positions did not correspond with those of the "rectal glands." He therefore fell into the serious error of announcing that the rectal gills could not be homologous with these latter structures, since they did not correspond with them in position. It will be one of the main purposes of this paper to show that the six main longitudinal folds in the *Simplex System* are the homologues of the six "rectal glands," and also to explain how it is that the more complicated *Duplex System* has apparently shifted its position. Sadones's error shows, indeed, how dangerous it is for a biologist to study the complex mechanism of the very highest term in a phylogenetic series, without any reference to, or knowledge of, those less specialized forms that hold the key to the situation.

The *Tracheal System* of the gill-basket is physiologically an *efferent* system, and may be considered as such in proposing the nomenclature for its parts. It must not, however, be forgotten that it was originally an *afferent* system, and is developed as such in the embryo. The efferent function begins almost immediately after the hatching of the young larva. It will be found, perhaps, simpler to follow the ramifications of this system as if it were an *afferent* system, *i. e.* starting from the main longitudinal tracheal trunks, and following the branches into the gills. The names, however, which we shall propose, will be such as are suggestive of the efferent function.

Starting then either from the great dorsal or the visceral trunks, a series of large tracheal branches may be seen branching off to the gill-basket. There are six of these series altogether, corresponding to the six rows of gills. These tracheæ are the *primary efferent tracheæ* of the gill-basket.

Arrived close to the outer surface of the gill-basket, each *primary efferent*

trachea divides up into two large branches, the *secondary efferent tracheæ*. These enter the gill-basket in twelve separate rows. Their methods of branching now vary according to the particular type of gill-basket which they are to supply. Their branches may be spoken of generally as the *gill-efferents* or simply the *gill-tracheæ*.

These gill-tracheæ branch and re-branch again within the gill-folds until they finally give off a very large number of very minute tracheæ, which form the last term in this tracheal series. These are the *capillaries*, *capillary loops*, or *tracheal loops* of the gills, by means of which the oxygen is received from the circumambient water in the rectum into the gills themselves. Not one of these tiny capillaries ends blindly; every single one is a complete *loop*, as Oustalet has clearly shown.

Terminology of the Various Types of Gill.

Five principal types of gill will be recognized in this paper. Of these, two belong to the *Simplex System* and three to the *Duplex*. Subordinate types will also be recognized under one at least of these principal types.

In the *Simplex System*, the two principal types are:—

1. The *Undulate Type*, in which the free edge of the gill-fold is thrown into complicated undulations or waves. (Text-figure 2.)

2. The *Papillate Type*, in which all except the basal portion of the gill-fold is split up into numerous long slender papillæ, each of which carries an *axial trachea* from which the capillaries arise. (Text-figure 3.)

In the *Duplex System*, the three principal types are:—

3. The *Implicate Type*, corresponding fairly closely to 1 in the *Simplex System*. In each hemibranch the gills are folded and grooved in a complicated manner, and the two series forming each holobranch lie so that their curved distal borders come into close relationship alternately with one another, and tend to overlap. (Text-figure 5.)

4. The *Foliate Type*, in which the transverse gills forming each hemibranch appear in the form of separate leaves or foliæ.

Under this type we may distinguish two very different sub-types:—

4 a. The *Normal Foliate Type*, in which each folia is undulated along its free edge, and bears no papillæ. (Text-figures 6-7.)

4 b. The *Papillo-Foliate Type*, in which each folia is folded over into a kind of oval "hump," bearing numerous tiny papillæ. These papillæ have no axial trachea, and only carry capillaries. (Contrast Type 2.) (Text-figure 9.)

5. The *Lamellate Type*, in which the gills forming each hemibranch appear as separate flat *lamellæ* or plates projecting into the rectum. (Text-figures 11-12.)

A considerable range of variation exists in the long series of forms which exhibit this type of gill. They do not, however, appear to be sufficiently distinct, or of sufficient importance, to warrant their elevation into the rank of sub-types of the same order as 4 *a* and 4 *b*, though I have suggested (p. 168) suitable names for them if it is desired to distinguish them in that way.

METHODS.

Three main methods of study have been followed, viz. :—

- (1) *The making of whole mounts of the branchial basket.*
- (2) *Photomicrography.*
- (3) *Microtomy.*

Whole Mounts.—Owing to the fact that the tracheæ in the gills collapse and lose their gaseous contents shortly after the death of the larva (usually in from one to two hours after death), it seemed at first that all attempts at making whole mounts must end in failure. However, after trying various methods without success, some very excellent results were obtained as follows :—

The larva was killed by being dropped into a tube of water in which a drop or two of chloroform had been well shaken up. It was then immediately dissected *under water*. The branchial basket, together with that part of the tracheal system surrounding it (including the main trunks), was dissected out, and opened up by a longitudinal cut near the mid-ventral line. It was then plunged at once into Carl's Fixative (see under *Microtomy*, p. 136), where it remained for 24 hours. After washing and dehydrating in the usual manner, it was passed into equal parts of absolute alcohol and cedar-oil for 24 hours, and thence into pure cedar-oil. There it remained for several days, during which time the gills darkened slightly. Finally, it was mounted on a slide within a raised ring, care being taken to remove as much cedar-oil as possible, so that the Canada Balsam should dry properly. The whole mount was then left for some weeks while it gradually darkened. The final result shows the gill-formation in yellowish-brown or orange, with the pigmented areas very strongly marked. The capillaries, of course, are not visible, as they have collapsed. But for a study of the *Simplex* or *Duplex System* in its entirety, these whole mounts are excellent. The drawings of portions of the gill-basket in the various systems (text-figs. 2, 5, 9, 11, 12) are taken from photographs of some of these mounts.

The everted gill-baskets are best mounted with their inner side uppermost, so that the whole gill-structure can be clearly seen. If it is desired to study the tracheal system, the slide may be turned carefully over and viewed from the back.

Photomicrography.—For this purpose the method followed previously by

Ris (23) was found to yield excellent results. The larva is killed in the manner shown above. As soon as it is dead, it is removed to a dissecting-dish and pinned *under water* with its ventral side uppermost. The dissection should not be made in glycerine or cedar-oil, since these highly refractive media are almost useless for the study of tracheal capillaries. The abdomen of the larva is opened by cutting away the projecting edges of the tergites and removing the sternites as a continuous ventral plate. The gill-basket can then be readily seen, lying in the posterior part of the abdomen. Next, the point of a pair of fine scissors is inserted into the posterior opening of the basket, and the latter is cut open longitudinally. This operation *everts* the gills, and the basket lies with its inner surface exposed. It is now easy to dissect out a complete holobranch. This should be placed on to a clean glass slide and floated out with a little water from a pipette. Having arranged it in a suitable position (*i. e.*, so as to obtain a good *lateral view* of it), a clean cover-glass should be allowed to descend lightly upon it. The gills are thus flattened out without being crushed or damaged, and the position of the tracheal capillaries is not disturbed. The gills may now be examined under a low or moderate power of the microscope, and a suitable portion selected for photography.

It is very necessary that the dissection, examination, and photographing of the gill should be completed within an hour or so of the death of the larva, because the air soon afterwards passes out of the capillaries, which then become invisible. There is no known method by which the air can be retained in these capillaries, so that permanent preparations can be obtained; and there is certainly no method that could yield results comparable to those obtainable by the method given, in which the capillaries stand out as black lines on a clear background.

The photomicrographic apparatus used was that of Reichert, Vienna, arranged in the vertical position. The photographs should be taken by transmitted artificial light (incandescent gas) on slow plates. With Ilford "Process" plates, which give excellent results, the exposure varies from fifteen seconds to four minutes, according to the magnification and the aperture of the iris diaphragm. In order to cover the plate it is best to use a No. 4 eyepiece. The most suitable objectives I found to be Nos. 1, 3, 6 *b*, and 8 *a*, giving magnifications of 30, 60, 320, and 560 diameters respectively with the closed tube. With the last-named objective a magnification of 725 diameters is obtainable by using the full length of the tube, and this can be extended to 850 diameters by lengthening the bellows of the camera. This last magnification is sufficient to show the complete structure of the smallest papillæ. One of the fine capillaries in a papilla of *Austrocordulia refracta*, Till., photographed at 850 diameters magnification, was found to measure barely 0.1 mm. in diameter. Its actual diameter in cross-section, therefore, would be about 0.12 μ .

Microtomy.—For this purpose two methods of procedure are available, both of which have their own peculiar advantages:—

1. The gill-basket may be dissected out as described above, and plunged at once into the fixative fluid. In the case of very complicated structures, such as the *Duplex System* of gill-basket in well-grown larvæ, some of the specimens should be dealt with by *everting* the basket with a longitudinal cut, so that the complicated foldings may be more widely separated.

2. The larva may be taken alive (preferably, soon after an ecdysis) and plunged into the fixative. By this means the fixative is drawn at once into the rectum by the movements of inspiration, and fixation of the rectum takes place from within.

The second method offers the advantage of studying the complete structure of the rectum in relation to the surrounding parts and body-wall. By it the very delicate rectal musculature is preserved entire, and the exact positions of the gill-eversions can be made out. But, before successful sectioning can be attempted, it is necessary to soften the chitinous integument of the larva, and this is a difficult task, if one would avoid any damage to the internal organs in consequence.

The first method enables us to proceed at once with dehydration and embedding, without the use of a softening process; but it only gives us, complete, the rectal structures enclosed within the circular muscle-layer. Hence it is advisable to obtain plenty of material and to use both methods, keeping the second only for use on larvæ which have just undergone ecdysis, and whose integument, in consequence, can be softened with the minimum of trouble.

Probably many methods of fixing, softening, and staining could be tried with success in the case before us. It seemed, however, advisable, bearing in mind the limited time at my disposal and the very great variety of material to be studied, to select one good method and to treat all the specimens alike. I therefore made use of the following method, previously applied in part by Carl and in full by Kurt-Bedau*, in studying the compound eyes of Insects:—

Fixation.—The fixative used was devised by Carl, and may be called Carl's Fixative. It consists of

Absolute alcohol	15 parts.	}
Concentrated formol	6 „	
Glacial acetic acid	2 „	
Distilled water	30 „	

The object should be left in this fixative for 24 hours, and then washed in 70 per cent. alcohol for 24 hours with several changes.

Softening.—If the second method of fixation, as given above, be used, the

* "Die Facettenauge der Wasserwanzen," Kurt-Bedau, Zeit. wiss. Zool., Bd. 97 (1910).

larva must now be placed for 24 hours or more (according to the state of hardness of the integument) in Soap Alcohol. This will extract all the fat, and occasionally causes local damage or malformation of a particular block of tissue; but it is a safe softening agent to use, provided it is not left in for too long a time. Hence the advice already given to soften only those larvæ which have recently undergone ecdysis. Soap Alcohol seems to me, in any case, preferable to Eau de Javelle.

Certain very tough larvæ, e.g. *Austrócordulia refracta*, seem to resist the softening treatment, even if immersed immediately after ecdysis. The integument of the larva is dark olive-green immediately after ecdysis, and hardens almost at once to a thick dark brownish state, which resists all attempts at softening within a reasonable time. In other larvæ the integument directly after ecdysis is very transparent and soft, and immersion in the soap alcohol need not be prolonged beyond a few hours.

The softening agent must be completely removed before dehydration by placing the larva again in 70 per cent. alcohol, and washing it in several changes extending over 24 hours or more. Before doing this, the abdomen should be cut off at about the 4th or 5th segment to allow of greater penetration on the part of the alcohol.

Dehydrating and Clearing.—The object should now be run up in the usual way through 90 per cent. alcohol (6 hours or less) to absolute alcohol (6 hours or less). It is then placed in a mixture of absolute alcohol and cedar-oil in equal parts for 24 hours. Finally, it is removed to pure cedar-oil and left there for 24 hours.

Infiltration and Embedding.—After clearing in cedar-oil, some finely sliced paraffin-wax of 36° M.P. (melting point) is introduced into the oil and allowed to dissolve. Enough should be added to make the mixture just pasty, so that, on warming ever so slightly, the fluidity returns again. The object is left thus for 24 hours. It is then placed in pure melted paraffin-wax of 36° M.P. for about 3 hours. From this it is passed into melted wax of 52° M.P. for a further 3 hours. Two or three changes may be given during each three hours in order to ensure the removal of all the cedar-oil. Finally, the object may be embedded in paraffin of 58° M.P. straight from the melted 52° M.P. wax; or, if the weather be warm, a further short period in melted wax of 58° M.P. may be given before the final embedding.

Sectioning.—As most of the gill-baskets are fairly large objects the majority of sections should be cut at 12 or 15 μ , the thickness being reduced to 5 or 7 μ at any desired level through a number of sections, in order to provide material for finer histological studies.

In the case of the whole larva the cutting should begin at the anal end, so that the rectum is reached as quickly as possible. The anal appendages can, of course, be removed with scissors before the softening process is begun—or after it, if preferred.

Double Embedding.—The ordinary single embedding method yields very satisfactory results in nearly all cases. For the papillate types, and whenever a very exhaustive histological analysis is required, celloidin embedding may be carried out from the pure cedar-oil stage as follows:—The object is embedded in celloidin in the usual manner, but with 1 part of cedar-oil added to 4 parts of the celloidin solution. On reaching the strongest celloidin solution the proportion of cedar-oil is reduced to 1 part in 5. The hardening is done in 5 parts of chloroform to 1 part of cedar-oil, the mixture being frequently changed. The block must then be embedded in paraffin dissolved in benzol to concentration in a closed vessel. The benzol must be allowed to evaporate very slowly for a week or more, until the paraffin is quite dry and firm. Sections may then be cut from the block as fine as 3 μ in thickness. The method is, however, not only a very protracted one, but offers considerable difficulties, especially in the successful elimination of the benzol.

MATERIAL STUDIED.

Nineteen species, belonging to fifteen genera, have been studied for the purpose of this paper. Microphotographs were taken of the gills of each of the nineteen species, and sets of serial sections prepared from the rectum of every genus represented. In some genera sections were obtained not only from the more advanced larval instars, but also from the earlier stages (first to eighth instars). The total number of sets of serial sections prepared and studied amounted to over seventy.

In the following table I have arranged the material studied in the accepted systematic order, giving family, subfamily, and tribe, together with columns indicating the system and type of gill to which each genus belongs:—

Family.	Subfamily.	Tribe.	Genus.	Species.	System.	Type.	
ÆSCHNIDÆ.	PETALURINÆ	<i>Petalura</i>	<i>P. gigantea</i> , Leach.	Simplex.	Undulate.	
	GOMPHINÆ	Gomphini	<i>Austrogomphus</i> ...	<i>A. ochraceus</i> , Selys.	"	"	
			<i>Hemigomphus</i> ...	<i>H. heteroclitus</i> , Selys.	"	Papillate.	
	ÆSCHNINÆ ...	Brachytronini ..	<i>Dendroæschna</i> ...	<i>D. conspersa</i> , Till.	Duplex.	Implicate.	
			<i>Austrophlebia</i> ...	<i>A. costalis</i> , Till.	"	"	
			<i>Austroæschna</i> ...	<i>A. multipunctata</i> , Martin.	"	"	
			<i>Æschna</i>	<i>Æ. brevistyla</i> , Ramb.	"	Foliate.	
		Æschnini	<i>Anax</i>	<i>A. papuensis</i> , Burm.	"	"	
	LIBELLULIDÆ.	CORDULINÆ...	Synthemini ...	<i>Synthemis</i>	<i>S. macrostigma</i> , Selys.	Duplex.	Lamellate.
			Idocorduliini	<i>Metathemis</i>	<i>S. eustalacta</i> , Burm.	"	"
<i>Austrocordulia</i> ...				<i>M. guttata</i> , Selys.	"	"	
Encorduliini ...			<i>Hemicordulia</i> ..	<i>A. refracta</i> , Till.	"	"	
			<i>Cordulephya</i>	<i>H. tau</i> , Selys.	"	"	
LIBELLULINÆ .		Libellulini	<i>Cordulephya</i>	<i>H. australia</i> , Ramb.	"	"	
			<i>Orthetrum</i>	<i>C. pygmaea</i> , Selys.	"	"	
		Sympetrini	<i>Orthetrum</i>	<i>O. caledonicum</i> , Br.	"	"	
			<i>Diplacodes</i>	<i>O. villos vittatum</i> , Br.	"	"	
				<i>D. hamatodes</i> , Burm.	"	"	
		<i>D. bipunctata</i> , Br.	"	"			

It will be noticed that a single type of gill, and that the most highly specialized of all, characterizes the whole of the Libellulidæ, while the other four types are distributed in a definite phylogenetic sequence within the Æschnidæ.

GENERAL STUDY OF THE BRANCHIAL BASKET.

A. *Its Position within the Abdomen.*

Longitudinal Extent.—When contracted the branchial basket occupies the whole length of segments 7 and 8, together with a small part of the posterior end of 6 and of the anterior end of 9. When expanded the gill-folds can be seen to reach forward as far as the middle of 6, or even a little further, and backwards nearly to the posterior end of 9. In length the gill-basket occupies three-fourths or more of the whole extent of the rectal region.

Position in Transverse Section (text-fig. 1).—The gill-basket, as seen in transverse sections of the abdomen, occupies a large, roughly circular space in the middle, surrounded by the hæmocœle. Dorsal to it lies the heart (*ht*), and immediately ventral to it is the ventral nerve-cord (*vg*). On either side it is flanked by the fat-body (*fb*) and a mass of tracheæ. Of these, the two large dorsal trunks (*dt*) lie dorso-laterally on either side, close to the gill-basket, while the smaller visceral trunks (*vst*) lie similarly ventro-laterally. Both dorsal and visceral trunks give off numerous branches, which enter the gill-basket. Each dorsal trunk gives off two sets of branches, each visceral trunk only one. The arrangement of these branches is discussed below (p. 181). The ventral trunks (*vnt*) lie at about the same level as the visceral trunks, but further apart, and send no branches to the gill-basket.

In the hæmocœle, between dorsal and visceral trunks, there may usually be seen two or three Malpighian tubules (*mlp*) running backwards from their point of origin at the anterior end of the proctodæal part of the intestine. Most of these tubules, however, lie well forward from the level of the gill-basket.

The other structures to be noted in a cross-section through the abdomen at this level are the large masses of the *segmental muscles*. These consist of dorsal or tergal longitudinal muscles (*mld*), ventral or sternal longitudinal muscles (*mlv*), and the smaller masses of the dorso-ventral or tergo-sternal transverse muscles (*mt*) occupying the angular spaces near the junction of the tergite with the sternite.

B. *Its Supports.*

The gill-basket, being part of the continuous alimentary tube, has no special supports of its own, unless its weak muscular tunic can be considered as such. But, just posterior to the gill-basket, and, in the later larval stages, also connected with the narrower posterior end of the basket itself, there can

be seen *six radial strands* of muscle arising in the folds of the thin epithelium separating the so-called "rectal glands," and passing out across the hæmocœle to become attached to the body-wall. These are the *dilatator muscles* of the rectum. They not only serve in a sense as actual supports for suspending the posterior part of the rectum firmly in the hæmocœle, but, by their contractions, cause the rectum to expand, and thus allow of the entry of water through the rectal valves into the gill-basket.

In the later larval stages the presence of these muscles gives the posterior portion of the gill-basket a characteristic hexagonal shape in cross-section. Further forward this effect is not noticeable, the usual shape of the basket being nearly circular or slightly compressed dorso-ventrally.

Using the clock-face notation, the positions of the insertions of the dilatator muscles are 1, 3, 5, 7, 9, 11. The positions of the rectal glands are 2, 4, 6, 8, 10, 12. These facts are of great importance when we come to the discussion of the homologies of the rectal gills.

C. *Its General Structure.*

All the gill-baskets of Anisopterid larvæ show the following general structure :—

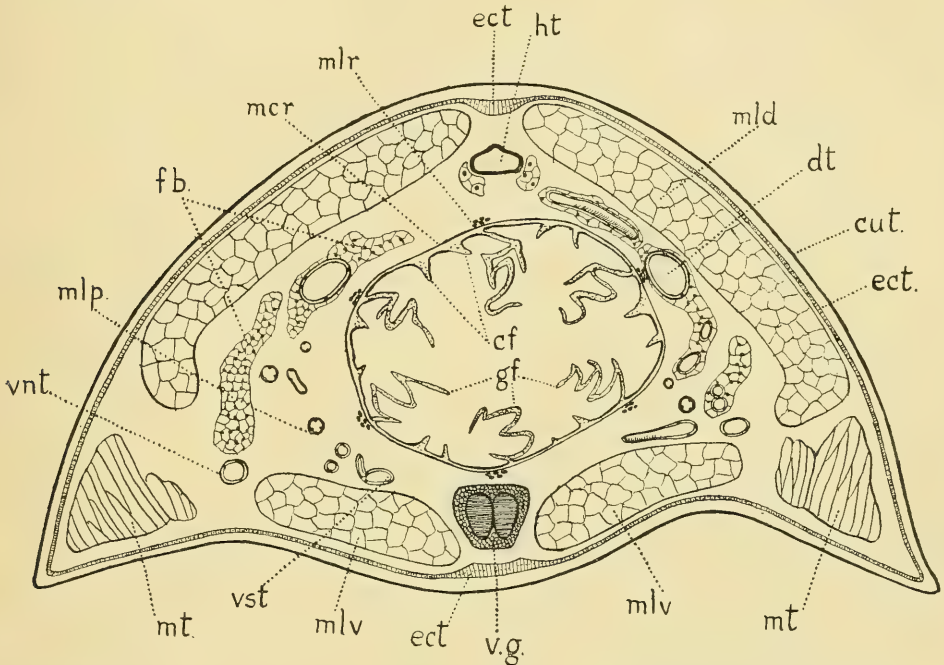
Passing from within outwards we meet first with a fine *cuticle* or *chitinous intima*. External to this is the *rectal epithelium*, from which the cuticle is derived. Surrounding the epithelium is an almost continuous layer of *circular muscle-fibres* only one row thick, with numerous openings for the entry of the tracheæ into the gill-basket. Finally, external to this, we can recognise a layer of longitudinal muscles, not forming a tunic, but segregated into six rather weak *longitudinal bands*, each formed of a small number of separate fibres. These are placed in the positions 2, 4, 6, 8, 10, 12 of the clock-face.

The Cuticle.—This is a very fine, transparent, non-cellular, non-staining layer which covers the internal face of the rectal epithelium throughout. It is absolutely smooth and structureless except along the edges of the gill-folds, where it is usually armed with a series of tiny chitinous teeth. It is secreted by the rectal epithelium, and is cast off at each ecdysis. It is exactly comparable with the similar fine internal cuticle which lines internally the stomatodæal portions of the alimentary canal.

The cuticle forms the outer covering of such organs as the papillæ and tubercles of the gills, when these are developed. In such cases it may develop a special armature of strong spines or of transverse chitinous rods, which will be described under the study of those types of gill in which it occurs.

The Rectal Epithelium.—This is remarkable in showing, throughout the gill-basket, no trace whatever of separate cell-divisions. It is, in fact, a

nucleated syncytium, and must be regarded as a highly specialized structure derived from an originally typical proctodæal epithelium with separate cell-boundaries. Except at certain definite places, where it swells up into the thick turgid structures known as the *basal pads*, this syncytium is a flat pavement-like layer containing numerous nuclei at fairly regular intervals. In some forms it is entirely unpigmented throughout; in others, especially at certain places, dense masses of pigment-granules are present and hide the nuclei from view.



TEXT-FIG. 1.—Transverse section through seventh abdominal segment of the larva of *Eschna brevistyla*, Ramb., 4th instar, to show position and structure of gill-basket. ($\times 32$.)

cf, cross-fold; *cut*, cuticle; *dt*, dorsal tracheal trunk; *ect*, ectoderm; *fb*, fat-body; *gf*, main gill-fold; *ht*, dorsal vessel or heart; *mcr*, circular muscle-layer of gill-basket; *mld*, dorsal longitudinal segmental muscle; *mlp*, Malpighian tubule; *mlr*, longitudinal muscle-band of gill-basket; *mlv*, ventral longitudinal segmental muscle; *mt*, transverse segmental muscle; *vg*, ganglion of ventral nerve-cord; *vnt*, ventral tracheal trunk; *vst*, visceral tracheal trunk.

The protoplasm of this syncytium is only very slightly chromatophil, giving only a light purplish stain after long immersion in hæmatoxylin. The nuclei, on the other hand, are very receptive to the same stain. They are of moderate size, usually rather oval, showing granular contents without any distinct nucleolus.

At regular intervals the rectal epithelium of the gill-basket is evaginated into the interior to form one of the *gill-folds*. Their position and extent vary in the different types of gill-basket; but the structure of every type of fold is essentially the same. The two walls of the evagination come into close apposition over their whole extent, except for a comparatively small basal portion, where they remain separate, enclosing a small space in which the *hypobranchial tissue* is developed, and into which the efferent or gill-tracheæ pass from the exterior (Plate 22. figs. 25-27). The fine tracheal branches or capillaries of the gill-fold are supported in the syncytium of one wall or the other. Throughout the distal portion of the gill, where the syncytia of the two walls fuse completely, the tracheal capillaries are seen to lie in the fused mass, but usually slightly nearer to one side than to the other. Owing to the great rapidity with which the air passes out from the capillaries after the death of the larva (no trace of air can be detected therein two to three hours after death) and to the absence of any spiral thread in these tiny tracheoles, it is seldom that one can make out the lumen of any of the capillaries in cross-section. But one finds, throughout the slender distal portion of the gill-folds, in addition to the already-mentioned nuclei of the epithelial matrix, a large number of tiny nuclei of very characteristic structure, which are undoubtedly the nuclei of the tracheal capillaries, since they correspond very closely to the nuclei lying in the matrix of the larger tracheæ. They are small, usually rounded, and stain deeply with hæmatoxylin, so that no very definite nuclear structure can be made out in them. Frequently one notices a tiny clear space attached to one side of one of these nuclei. This appears to me to represent the collapsed lumen of the capillary vessel. Other small bodies noticeable in the syncytium of the distal portion of the gill, but not present in the gills of all forms examined, are small pigment-granules and tiny transparent globules of a highly refractive nature, probably composed of fat.

The basal portion of each gill-fold, as has been already stated, broadens out so that a space of greater or less extent is formed between the two walls. Here the epithelium either of the anterior of the two walls, or of both, may be broadened out into the form of a thickened *basal pad*. As these structures vary much in shape and position, as well as in actual size and thickness, I have postponed a general discussion of them until after the various types of gill have been described (see p. 170). For the same reason I shall here only mention the presence, in the space between the walls at the base of a gill, of a peculiar mass of tissue whose origin and functions seem to be rather doubtful. This is the tissue called by Chun and Faussek *connective tissue*, by Sadones *adipose tissue*. As both these names are admittedly unsuitable, I propose here for it the name *hypobranchial tissue*. We may then define the hypobranchial tissue of the gill-basket of Anisoptera larvæ as the mass of tissue lying in the basal space between the two walls of the gill-fold, and

carrying in its meshes the efferent trachea and its main branches for that particular portion of the gill-fold under consideration. Generally speaking, the *hypobranchial tissue* is flanked on one or both sides by the basal pad, from which it is easily distinguished at once by its different structure, nuclei, and staining qualities (see p. 176).

The Circular Muscle Tunic.—The outstanding feature of the circular muscle-layer of the gill-basket is its weakness. It is everywhere composed of only one row of fibres, which run closely parallel around the basket so as to form a complete investment, except for numerous spaces left for the tracheæ to pass through (text-fig. 1, *mc*r). The weakness of this tunic precludes the supposition that it is of use in the contraction or expansion of the gill-basket. These movements appear to be caused by the movements of the segmental muscles of the abdomen, and in particular by the six *radial* muscles already mentioned.

The Longitudinal Muscle-Bands.—In the young larva each longitudinal band is seen to be composed only of five or six strands of muscle-fibre placed fairly close together so as to form a definite band (text-fig. 1, *ml*r). In more mature larvæ a larger number of fibres is present, and the longitudinal bands become very definite structures. They do not seem, however, to be of much strength considering the size of the gill-basket. They aid, by their contractions, the shortening of that organ which takes place regularly during the act of rectal breathing; but their function can only be regarded as auxiliary to that of the far more powerful segmental muscles of the abdomen.

STUDY OF GILL-TYPES.

A. SIMPLEX SYSTEM.

Definition.—The *Simplex System* of gills consists of six holobranchs in the positions 2, 4, 6, 8, 10, 12 of the clock-face. Each holobranch is a continuous longitudinal eversion of the rectal wall, supported to right and left alternately by numerous small *cross-folds*, these latter lying in a slanting direction nearly transverse to the main fold, and not projecting far into the rectal cavity.

The Tracheal Supply.—We have already mentioned that each *primary efferent trachea* divides into two *secondary efferent tracheæ* before reaching the branchial basket. Thus there are *twelve sets* of *secondary efferent tracheæ* entering the basket from outside. There are also *twelve sets* of cross-folds, viz., one set to right and one set to left of each main longitudinal fold. Now these cross-folds lie below the courses of the secondary tracheæ—in fact, each cross-fold is developed in relation to a single secondary trachea, which sends out branches and capillaries into it. Each secondary trachea, however, continues inwards in a slanting direction until it reaches a main longitudinal fold, into which it gives off branches and, finally, capillaries.

In order to understand the somewhat complicated manner in which the tracheæ are distributed, let us look at the diagram in Plate 22. fig. 22. We see from this that the *primary* efferent tracheæ approach the circumference of the gill-basket at the positions 1, 3, 5, 7, 9, 11 on the clock-face, though the main gill-folds, as already stated, lie in the positions 2, 4, 6, 8, 10, 12. The *secondary* efferent tracheæ, formed by the bifurcation of any given primary trachea, enter the basket therefore slightly to right and left of one of the positions represented by an *odd* number on the clock-face. Nor do the two secondary tracheæ from any given primary trachea enter at the same *transverse* level—one is always a little in front of the other. This is shown in the diagram by representing these tracheæ by alternate complete and dotted lines, the dotted ones not being at the same level as the complete ones.

After giving off branches to the cross-folds, the secondary tracheæ run on to enter the main folds at their bases. This they do by branching into a series of gill-tracheæ, which spread out at different levels, and then bend sharply inwards to enter the main folds. Each series of gill-tracheæ forms a small *fan* or pencil of rays, and enters the main fold at a convexity, or crest, of the undulations of its base-line.

It follows from this that each main longitudinal fold receives tracheæ from two sources which supply it alternately, viz., a series entering from the right and one from the left. In the case of the mid-dorsal longitudinal gill-fold (position 12) the series to the right is a set of gill-tracheæ given off by the series of secondary efferent tracheæ which form the more dorsal or left-hand branchings of the upper series of primary efferents given off by the *right* longitudinal dorsal tracheal trunk; while the series to the left is derived correspondingly from the upper series of primary efferents given off by the left dorsal trunk. Thus the gill in position 12 receives its tracheæ alternately from the positions 1 and 11, or thereabouts. A similar arrangement holds for each of the other five main gill-folds.

The main longitudinal gill-folds are older, both phylogenetically and ontogenetically, than the cross-folds (see p. 187), and are the only gills present in the rectum in the newly-hatched larva.

The cross-folds are never very large, nor do they project far into the rectum. They are always very crumpled and irregular. As they are well supplied with tracheæ, they certainly aid in respiration. Probably the twelve sets of cross-folds taken together scarcely extract more than 50 per cent. of the amount of oxygen extracted by the six main folds in the same period of time.

The principal interest of the cross-folds is their relationship to the gills in the *Duplex System*, to be dealt with later (p. 150).

We shall now turn to the study of the two types of gill known to exist, which belong to the *Simplex System*. These are (1) the *Undulate Type*,

(2) the *Papillate Type*. A careful study of these two types will give us a much clearer idea of what the *Simplex System* is really like.

1. **Undulate Type.** (Text-fig. 2 ; Plate 18. fig. 1 ; Plate 21. fig. 17 ; Plate 22. figs. 22, 25.)

This type has been found by me in the gills of *Petalura* and *Austrogomphus* (text-fig. 2). Dr. Ris has also found it in *Cordulegaster*. It therefore occurs just in those genera which are already acknowledged, from a study of other characteristics, to be the most archaic of living Anisopterid dragonflies. The principal characteristic of the type is that each main



TEXT-FIG. 2.—*Austrogomphus ochraceus*, Selys.

Posterior portions of three holobranchs from the everted gill-basket of a full-grown larva. *Undulate Type*. Semi-diagrammatic, drawn from a whole mount. ($\times 30$.)

longitudinal gill-fold is thrown into fairly regular folds or undulations. These folds do not stand directly out into the rectal cavity, normal to the circumference, but slant away posteriad, and lie so close to one another that they give the appearance of a somewhat complicated frill with its folds all running towards the posterior end of the rectum (text-fig. 2).

The cross-folds are of the same folded or undulate type, but much less prominent, and appear to be more crumpled and irregular. Their position in relation to the main folds is well shown in text-fig. 2.

Along the free borders of the folds the cuticle carries a series of tiny chitinous hooks placed at wide intervals apart and directed backwards. These are scarcely visible at all in *Austrogomphus*, but can be clearly made out in *Petalura* under a high power.

Arrangement of the Tracheæ in the Gill.

Plate 18. fig. 1 shows the arrangement of the tracheæ in a portion of a main longitudinal gill-fold of *Austrogomphus ochraceus*, which, previous to being photographed, had been smoothed out almost into one plane by means of a camel's-hair brush. The gill-tracheæ leave the secondary efferents along the line of the base of the gill a little to left and right of it alternately. Each undulation is usually supplied by four or five of these gill-tracheæ, which are of gross calibre run far up into the gill-fold, their distal ends reaching fairly close up to the free edge of the fold. From each gill-trachea short side-branches are given off, most abundantly towards its distal end. These branches quickly break up into tufts or bundles of capillaries, which also seem to be most numerous towards the distal ends of the gill-tracheæ. The capillaries diverge as they leave the branches, and spread out all over the gill-fold, running slantwise at all angles towards the free edge of the gill. Here they all turn over *in complete loops*. It is possible under a high power, by careful manipulation of the fine adjustment, to follow the course of a single capillary, and to note two interesting facts in connection with it. First, those capillaries which arise near the distal end of a gill-trachea, after forming their loops, run back to enter branches of an adjoining gill-trachea which are situated more proximally to the gill-base. Secondly, those capillaries which arise so as to run along one gill-wall, after forming their loops, descend along the opposite gill-wall. As the two gill-walls are in very close apposition, this change of position can only be accurately judged by the use of the fine adjustment. By examining the free edge of the gill-fold in Plate 18. fig. 1, about the middle of the picture, both the above conditions can be made out fairly well.

In the cross-folds, the gill-tracheæ, short branches, and capillaries are very similar to those in the main folds, but the gill-tracheæ are shorter, and do not extend so far into them. They are thus mainly filled with capillaries which arise and end more at the same level than do those in the main fold. In Plate 18. fig. 1, three cross-folds can be seen flattened down below the main fold, while portions of two intermediate cross-folds lying above the main fold can also be made out.

Number of Capillaries in the Gill-basket.

In *Austrogomphus ochraceus* I estimate the number of complete loops belonging to each secondary efferent trachea at 100 or over. There are 6 longitudinal main folds, each of which is thrown into about 12 principal undulations, each of which receives approximately the whole of the capillaries from one secondary efferent trachea and its branches. Thus we get a total of $100 \times 6 \times 12$, or 7200 complete capillary loops for the six main folds. Allowing a total of 50 per cent. extra capillaries for the twelve

sets of cross-folds, whose total area probably does not exceed one-half of the total area of the six main folds, we thus obtain an approximate total of 10,800 complete capillary loops in the whole gill-basket.

In *Petalura gigantea*, a larva which is many times larger than that of *Austrogomphus ochraceus*, the gill-tracheæ are not only much shorter, but more numerous. As the only larvæ available for examination had been preserved for four years in alcohol, none of the capillaries were visible, and it was therefore, of course, quite impossible to attempt an estimate of the number of finer capillaries. We should, I think, be well under the estimate in assigning a total of 20,000 complete loops to this large larva, which even then would be poorly supplied in comparison with the much smaller *Austrogomphus* larva, after making due allowance for its much greater size.

In *Cordulegaster*, the gill-folds resemble those of *Petalura* very closely in the thickness and arrangement of the efferent trachea and the firmness of the cuticle.

The occurrence of the *Undulate Type* in *Austrogomphus* is very remarkable, since all other *Gomphineæ* whose larvæ have so far been examined have been found to possess the *papillate* type of gill. The discovery of this important difference, then, suggests that the genus *Austrogomphus* is not closely related to any of the Palæarctic genera, and is very probably an isolated remnant of an even older Gomphine fauna.

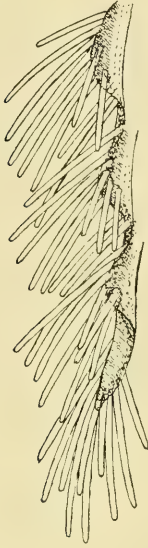
It should, however, be borne in mind that none of the larvæ of the larger *Gomphineæ* of the tribe *Ictini* have yet been studied in this connection. In these larger, less reduced, and presumably more archaic genera, we may well hazard the supposition that some at least, if not all of them, will be found to possess the more primitive *undulate type* of gill.

2. Papillate Type. (Text-fig. 3 ; Plate 18. fig. 2.)

This type of gill has been found by me only in the larva of *Hemigomphus heteroclitus* (text-fig. 3). Dr. Ris has shown it to exist also in the larvæ of the Palæarctic Gomphine genera *Gomphus* and *Onychogomphus*. It seems to be characteristic of the main mass of the *Gomphineæ*—or, at any rate, of the tribe *Gomphini*.

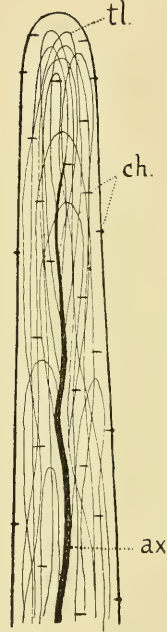
As in the *Undulate Type* of gill-basket, each of the six main folds is a complete longitudinal eversion of the rectal wall, with supporting cross-folds arranged in the same manner. But, in the present case, these complete eversions of the wall only project a very short distance into the cavity, and carry along their distal border a very conspicuous layer of dark purplish-brown pigment. Below this layer of pigment each gill-trachea is found to split up into a set of five or six branches. Each of these branches projects *through* the pigment-layer, carrying with it the wall of the rectum, so as to form a long slender papilla of cylindrical form, projecting far into the

rectum, and having the tracheal branch as a kind of axis within it. These branches of the gill-tracheæ will be called the *axial* or *papillar tracheæ* (text-fig. 4, *ax*). The papillæ, though actually arising thus in groups of five or six, each group corresponding to a single "wave" in the original undulate type, are so numerous and closely set as to appear like a continuous series. The papillæ are developed, not only along the course of the main gill-folds, but also along the cross-folds. Hence, on opening the gill-basket longitudinally, one sees a mass of papillæ of an intense shining whiteness, projecting apparently from all points of the surface.



TEXT-FIG. 3.—*Hemigomphus heteroclitus*, Selys.

Posterior portion of a main fold from the everted gill-basket of a nearly full-grown larva, viewed somewhat in profile. Cross-folds omitted. *Papillate Type*. Semi-diagrammatic, drawn from the freshly opened gill-basket. ($\times 30$)



TEXT-FIG. 4.—*Hemigomphus heteroclitus*, Selys.

Anterior portion (about one-fourth) of a papilla from the same larva. ($\times 600$) *ax*, axial or papillar trachea; *ch*, chitinous rods; *tl*, loops of tracheal capillaries.

Structure of the Papilla.—Each papilla consists of a simple finger-like eversion of the wall of the gill-fold, containing no pigment. Its chitinous cuticle, when examined under a high power, is seen to be beset with numerous short transverse chitinous rods, which give it a finely ribbed appearance (text-fig. 4, *ch*). The use of these little chitinous projections seems to be to prevent the papillæ from clinging together, and thus to

ensure a complete circulation of aerated water around each single one of them. [Compare the function of the tubercles found on the lamellæ of Libellulidæ, for which see p. 162.]

Arrangement of the Tracheæ.—We have already shown how the separate gill-tracheæ divide each into five or six axial or papillar tracheæ, each of which then forms the axis of a separate papilla. The capillaries are almost entirely confined to the papillæ, only a few stray ones being detectable here and there in the pigment-layer. Very possibly these may have become displaced or pulled out from the bases of papillæ during the process of dissection. The capillaries arise along the whole course of the papillar trachea. They are of excessive fineness, and can only be clearly detected under a high power (text-fig. 4, *tl*). They run almost parallel to the main axis of the papilla. But, whereas the papillar trachea itself does not reach the extreme tip of the papilla, all the capillaries, on the other hand, run right up to the very tip, where they curve sharply over in small loops and run back to rejoin the papillar trachea near its distal end.

In the everted gill-basket, the papillæ are seen to project in a slanting position directed somewhat posteriorly. The angle between the papillar axes and the longitudinal axis of the gill-fold varies between 130° and 140° , being greatest at the posterior end of the gill-basket, where it terminates in a series of long papillæ projecting beyond the end of the basal fold.

Origin of the Papillate Type.—The type seems to have been called forth in the course of evolution by the assumption of peculiar habits on the part of the larva. Whereas the larvæ of the genus *Austrogomphus* all live on the river-bottoms, hidden in loose débris, or buried only partially so that their heads and anal ends project freely into the water, the larvæ of *Hemigomphus*, on the other hand, bury themselves completely in pure sand well below the surface of the river-bed. In this they burrow or remain motionless all day, only emerging at night to look for food. The anal opening is protected, as in *Petalura*, not only by valves, but by crossed hairs, which allow of the inspiration of water from the damp sand, while keeping the sand itself out. When the larva is buried in the sand, the process of respiration must necessarily be very slow, so that it is of the utmost importance that the larva should command the most efficient and complete extraction of oxygen from the small quantity of available water. This objective is certainly marvellously well ensured by the papillate type of gill, which can be easily seen to have two very distinct advantages over the undulate type:—

1. As a result of the splitting-up of the gill-tracheæ, a very much greater number of capillaries is developed.

2. As the capillaries lie in groups isolated in separate papillæ projecting far into the rectal cavity, their ability to absorb oxygen is very much increased, especially as the water bathes each papilla on all sides, whereas in the undulate type it can only course along the two sides of each fold.

Number of Capillaries in the Gill-basket.

It is not easy to estimate the number of capillaries in a papilla. They are sufficiently numerous to give a *striated* appearance to the papilla; but the diameter of the latter is very narrow, and hence I have placed the minimum number at 20 complete loops. There are about 100 papillæ developed along a single longitudinal fold in *Hemigomphus*. Hence we get a total of $6 \times 100 \times 20$, or 12,000 complete loops for the six folds. Adding 50 per cent. for the cross-folds, we obtain a total of 18,000 loops as a conservative estimate. This is a great increase in the number estimated for *Austrogomphus* (10,800), especially as the larvæ of *Hemigomphus* examined were not full-grown and were of smaller size than those of *Austrogomphus*.

Considering that it belongs to the *Simplex System*, the papillate type must be given a very high place in the development series for the remarkable level of efficiency attained by it. Judging also by other attempts made in the course of evolution to develop papillæ on much more highly developed gill-folds of the *Duplex System*, we must regard these devices as being amongst the most efficacious yet evolved for the extraction of oxygen from the surrounding water.

B. DUPLEX SYSTEM.

Definition.—The *Duplex System* of gills consists of six double holobranchs whose middle lines or axes of symmetry lie in the positions 1, 3, 5, 7, 9, 11 of the clock-face. Each holobranch consists of two hemibranchs possessing a tracheal supply derived from a single series of primary efferents. Each hemibranch consists of *a series of separate gills* arranged in a direction more or less transverse to the middle axis of the holobranch, and corresponding, in position and development, with the series of cross-folds already mentioned in the *Simplex System*. Main longitudinal folds, of the type and position defined in the *Simplex System*, are either completely absent, or, if present, are aborted and do not carry tracheæ.

The Tracheal Supply.—Although the Duplex System seems at first sight to be so much more complicated than the Simplex, yet its tracheal supply is much more easily understood. For, with the elimination of the main longitudinal folds, the Duplex System may be seen to resemble the *cross-folds alone* of the Simplex System. Thus, then, each series of primary efferent tracheæ (approaching the gill-basket at the positions 1, 3, 5, 7, 9, 11 of the clock-face, respectively) supplies a complete holobranch in a corresponding position. When a primary efferent bifurcates, one branch, or secondary efferent, goes to one hemibranch, the other to the other. So, then, each of the twelve hemibranchs is *completely* supplied by tracheæ from a single complete series of secondary efferents.

To give an example :—The more dorsal, or left-hand hemibranch of the

holobranch in position 1 is supplied by the more dorsal or left-hand series of secondary efferents arising from the upper set of primary efferents given off by the right dorsal longitudinal tracheal trunk.

The diagrams in Plate 22, figs. 23, 24, show the method of tracheal supply, and should be carefully compared with that for the Simplex System (Plate 22, fig. 22).

We shall now turn to the study of the three main types of gill into which the Duplex System may be divided, viz. (1) the *Implicate*, (2) the *Foliate*, and (3) the *Lamellate*.

1. *Implicate Type*. (Text-fig. 5; Plate 18, figs. 3, 4; Plate 21, fig. 18.)

This type of gill has been found by me in larvæ of the genera *Dendroæschna*, *Austrophlebia*, and *Austroæschna*. Dr. Ris has also found it in the Palearctic genus *Brachytron*. All these genera belong to the tribe *Brachytronini* of the subfamily *Æschnini*. Hence we may fairly claim the *Implicate Type* as characteristic of, and peculiar to, this tribe of dragonflies.



TEXT-FIG. 5.—*Austroæschna multipunctata*, Martin.

Posterior two-thirds of three hemibranchs from the everted gill-basket of a full-grown larva. *Implicate Type*. Semi-diagrammatic, drawn from a whole mount. ($\times 30$.)

characteristics, but taking on a special shape and arrangement of their own. The complicated formation resulting from this may be well studied in text-fig. 5. From this figure it will be seen that each separate gill projects from its base-

line in a slanting direction, so that its rounded and free distal end comes to overlap a corresponding gill from the next consecutive hemibranch situated slightly more posteriad. The two sets of overlapping gills belong to one complete holobranch. Further, each gill is in the form of an elongate-oval leaf on a broad base, with its upper surface very concave, giving it almost a grooved appearance. Also, the free border of each leaf is waved or undulated, sometimes only moderately as in *Austroeschna*, sometimes very deeply as in *Dendroeschna*. In the latter case, the complicated appearance of the newly everted gill-basket almost defies description.

Dr. Ris's short account of the gills in *Brachytron* show that they resemble very closely those of *Austroeschna*. He says that they are "leaves which project like tiles one over another" ("Blätter, die sich dachziegelförmig überlagern").

There are some important differences to be noted in the gills of the four genera here under consideration. In *Dendroeschna* and *Austrophlebia* there is no sign either of fat-globules or of pigment, and the whole apparatus is of a pure shining white colour. The frills along the free borders of the gills are very numerous and complicated, especially in *Dendroeschna*, where the whole gill-formation resembles nothing so much as a turbulent sea of waves and wavelets inextricably intermingled. Transverse sections of the rectum of this larva reveal the almost unbelievable extent to which this complex folding and frilling is carried.

In the genera *Austroeschna* and *Brachytron* there is no complicated frilling of the edges of the gills. They are, instead, fairly regularly waved and undulated. There is a considerable amount of pigmentation of a purplish-brown colour.

It is interesting to note that in the genus *Austroeschna* there is a distinct tendency shown, in the posterior region of the gill-basket, towards a constriction of the broad bases of the gills. Thus the gills in this part of the rectum tend to approach the *Foliate Type* as developed in the genus *Æschna* (p. 153). If we now also bear in mind the fact that it is just in this posterior region of the gill-basket, in *Æschna* itself, where the gill-foliæ are most widely separated off and most definitely developed, we may well claim to have here definite evidence that the earliest form of *Implicate Type* gave origin to the *Foliate Type* during the course of evolution.

Arrangement of the Tracheæ.—Each separate gill is supplied by a strong thick secondary efferent trachea, which approaches its base from the side nearest to the middle line of the holobranch to which it belongs. This secondary efferent bends sharply down into the base of the gill, branching into several stout gill-tracheæ. These run straight up towards the distal border of the gill, giving off numerous capillaries and also smaller branches which also quickly break up into capillaries. The structure of the free border and the arrangement of the capillaries resemble very closely those

of the *Undulate Type* of gill. The capillaries are very numerous and vary somewhat in fineness. In *Austroæschna* and *Brachytron* they seem to be much of the same calibre as in *Cordulegaster*, but in *Dendroæschna* and *Austrophlebia* they are of excessive fineness and very difficult to photograph. In these genera, too, there is a more extensive branching of the gill-tracheæ, so that each "frill" is supplied more or less completely with capillaries thrown out from a single branch.

Number of Capillaries in the Gill-basket.

In *Austroæschna* we should assign about 200 complete capillary loops to each secondary efferent trachea (inclusive of its large branches). Each hemibranch receives, on an average, 12 of these sets. Hence the total number of complete loops is $12 \times 12 \times 200$, or 28,800.

In *Dendroæschna*, it is almost impossible to attempt an approximation. Each "frill" must carry a minimum of at least 20 loops. The edge of each groove or plication carries 16 or more "frills," and there are 12 grooves to a hemibranch. Thus we get a total of $12 \times 12 \times 20 \times 16$, or 46,080 complete loops in the gill-basket! The much larger larva of *Austrophlebia* probably contains an even greater total. The very great increase in the number of loops in these genera, compared with *Austroæschna* and *Brachytron*, is probably correlated with their habits of hiding under rocks in fast mountain-streams, where the act of inspiration is perhaps difficult and intermittent.

2. Foliate Type.

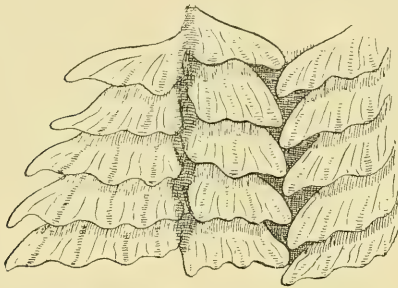
We now pass on to a very interesting type of gill, found only, so far as is known, in the two large and widely distributed genera *Æschna* and *Anax*, both belonging to the tribe *Æschnini* of the subfamily *Æschninae*. Of these two genera, *Æschna* alone exhibits the normal or typical foliate gill-form. In *Anax* there is an additional complication in the form of an abundant development of small papillæ. We must, therefore, subdivide the foliate type into two sub-types—the *normal foliate* type of *Æschna*, and the *papillo-foliate* type of *Anax*.

2 A. Normal Foliate Sub-type. (Text-figs. 6-7 ; Plate 19. figs. 5-6 ; Plate 21. fig. 15 ; Plate 22. figs. 23, 26.)

This sub-type has been studied by me in *Æschna brevistyla*, and appears to agree very closely with the form of gill found in the Palearctic members of the same genus. In it the gills of each hemibranch form a series of leaves with narrowed stalks and broadly expanded distal ends. The secondary efferent trachea forms the axis of each stalk, while the capillaries are confined to the expanded leaf. On entering the base of the leaf the

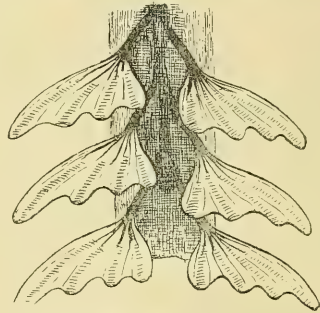
secondary efferent trachea gives off branches, which branch again and again until the whole leaf is supplied with an immense number of capillaries. The free edge of each leaf is much folded in and out, giving it the appearance seen in a crinkled rhubarb or cabbage leaf. Fat-globules and dense purplish-brown pigment are plentifully distributed, not only in the basal swellings of the rectal epithelium, but also in the leaves themselves. The thickness and complexity of the formation, together with the dense pigmentation, make it impossible to do justice to its appearance in a photograph.

The leaves or *foliæ* arising in two longitudinal rows from one holobranch tend to diverge distally. But the *foliæ* from right and left halves of two consecutive holobranchs do not come into very close contact, because each *folia* assumes a direction nearly transverse to the long axis of the gill-basket. Thus all the *foliæ* come to project into the cavity at approximately equal intervals apart, the distal end or tip of each lying so as just to interpose



TEXT-FIG. 6.—*Æschna brevistyla*, Ramb.

Portions of three hemibranchs from the anterior portion of the everted gill-basket of a larva at about the 10th instar. *Normal Foliate Sub-type*. Semi-diagrammatic, drawn from the freshly-opened gill-basket. ($\times 50$.)



TEXT-FIG. 7.—*Æschna brevistyla*, Ramb.

Portions of two hemibranchs from the posterior narrow portion of the gill-basket of the same larva. *Normal Foliate Sub-type*. Semi-diagrammatic, drawn from the freshly-opened gill-basket. ($\times 50$.)

itself into the space left between two consecutive *foliæ* of the next hemibranch (text-fig. 6). Hence, on everting a gill-basket of *Æschna* by a longitudinal cut, one notices at first twelve rows of leaves arranged in six sets in which the distal ends of the leaves face one another, while the tracheal axes appear to diverge from the region of a longitudinal axis which is the true axis of symmetry of each holobranch. The two hemibranchs really belonging to one holobranch have their *foliæ* arranged so as to diverge away from one another distally.

Arrangement of the Tracheæ.—If one of the *foliæ* be dissected out, and its free border smoothed out by means of a camel's-hair brush, it will be seen

that the arrangement of the tracheæ is very similar to that already seen in *Austroeschna*. There is, however, a more complicated branching of the tracheæ, and the finer branches run very close up to the border of the leaf, giving off numerous fine capillaries which arch along the free border of the leaf, and then turn over downwards to join other branches. These latter unite up, finally, to enter the main trachea of the gill at a different level. Thus the whole folia, but especially its free distal border, is richly supplied with capillaries.

Differentiation of the Posterior Portion of the Gill-basket.—In the gill-basket of *Æschna* there is a very large and broad anterior portion followed by a much smaller and narrower posterior portion. On everting the basket by a longitudinal cut, it will be seen at once that there is a difference in the arrangement of the foliæ in the anterior and posterior portions. In the anterior portion the foliæ are closely crowded together, the spaces between succeeding foliæ in a hemibranch being very short. In the posterior portion, however, the number of foliæ is very small; they are exceedingly well developed, and seem to lie more freely in the rectal cavity. Also they are separated from one another by much wider intervals. Text-fig. 6 shows the arrangement of the foliæ in the anterior part, while text-fig. 7 shows some foliæ from the posterior part.

Presence of Aborted Main Longitudinal Folds.—In order to show these clearly, I fixed and cleared the whole abdomen of a well-grown larva of *Æschna brevistyla*. While in cedar-oil, this was cut into thick transverse sections (2 mm.) by means of a sharp razor. On examining these sections under a low power, the six main longitudinal folds developed in the *Simplex System* of the young larva (see p. 181) could be clearly made out, separating the double rows of hemibranchs of the *Duplex System* of the more mature larva. They are devoid of dark pigmentation, and carry no tracheæ. They may be followed right back to the posterior end of the gill-basket, where they pass into the six corresponding "rectal glands." The small posterior terminations of the gill-hemibranchs may be actually seen in the same section as the "rectal glands," at the posterior end of the gill-basket, which in this genus passes, without any sphincterial separation, into the anal portion of the rectum (text-fig. 8).

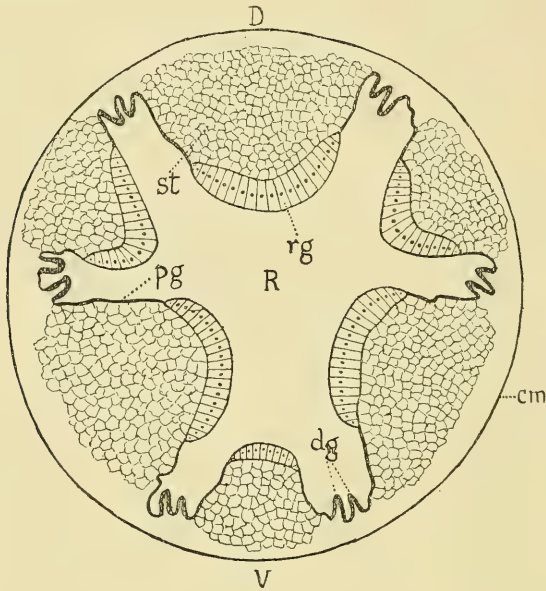
The study of this genus, then, makes it quite certain that the main longitudinal gill-folds of the *Simplex System* are actually homologous with the so-called "rectal glands," with which they agree both in number and position.

Number of Capillaries in the Gill-basket.

In *Æschna brevistyla* there are 16 foliæ in a hemibranch (rarely 17 or 18). In each folia there are 8 or more branches of the main tracheal stalk, and each branch may be credited with a minimum of 50 capillary loops. Hence

we get a total of $12 \times 16 \times 8 \times 50$ or 76,800 complete capillary loops in the gill-basket! This enormous number, together with the great increase in pigmentation, represents a very distinct evolutionary advance over the Implicate Type.

Origin of the Foliate Type.—This type is easily derivable from a simple form of the Implicate Type. If each gill in the latter type were to become constricted off below its middle, it would quickly take the form of a separate leaf, supported on a stalk in which the efferent trachea ran. The “frills”



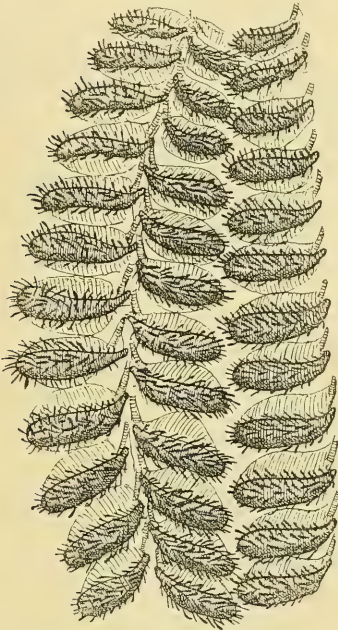
TEXT-FIG. 8.—*Aeschna brevistyla*, Ramb.

Transverse section through extreme posterior end of gill-basket, showing the reduced and highly-pigmented gills lying between the so-called “rectal glands.” Section cut from a larva which had just completed its ecdysis into last larval instar. Semi-diagrammatic. ($\times 72$) *cm*, circular muscle; *D*, mid-dorsal position; *dg*, duplex gill-system; *pg*, pigmented rectal epithelium; *R*, rectum; *rg*, raised epithelium of “rectal gland”; *st*, supporting-tissue; *V*, mid-ventral position.

on the edge or “lip” of the groove would then correspond to the “crinkles” on the leaf. Finally, if each separated groove became elongated transversely to the longitudinal axis of the gill-basket, and came to lie more transversely to that axis, we should then obtain a strictly foliate type of gill-structure, only differing by the absence of fat-globules and dark pigmentation from the foliate gill of *Aeschna*.

2 B. **Papillo-Foliate Sub-type.** (Text-figs. 9-10; Plate 19. figs. 7-8; Plate 21. figs. 16, 19.)

The very remarkable form of foliate gill-type found in *Anax* has been studied by Dufour, Oustalet, and others. All of these authors up to Poletaiëw believed that this type of gill belonged to the genus *Æschna*, but none of them attempted to breed out the larva which they were studying. Poletaiëw, who studied undoubted larvæ of *Æschna*, failed to find the



TEXT-FIG. 9.—*Anax papuensis*,
Burm.

Three complete hemibranchs from the everted gill-basket of a full-grown larva. *Papillo-foliate Sub-type.* Semi-diagrammatic, drawn from a whole mount. ($\times 40$.)



TEXT-FIG. 10.—*Anax papuensis*,
Burm.

A single papilla from the same larva. ($\times 360$.) *b*, base; *ch*, chitinous hooks; *pg*, pigmented epithelium of the basal hump; *tl*, loops of tracheal capillaries.

papillæ mentioned by these earlier authors. She therefore questioned the accuracy of their descriptions, but failed to discern the true cause of the discrepancy. It remained, then, for Ris to show that the truly *papillo-foliate* gill belonged to the genus *Anax*. My own studies of this type have been carried out on the larva of *Anax papuensis*.

The structure of the separate leaves in *Anax* is very easily understood, in spite of their complexity. Each leaf, instead of being of the crinkly cabbage-

leaf form seen in *Æschna*, forms a transversely-elongated oval hump, borne upon a slender stalk whose axis is a secondary efferent trachea. It is this character of the *narrow stalk* which is typical of the foliate type, and distinguishes it from all others. In *Anax*, these leaf-stalks are not quite so long as in *Æschna*, and consequently the leaves do not project so far into the rectum. There are twelve leaves or humps in each row in *Anax papuensis*. Rarely, thirteen or fourteen occur. The positions of the leaves or humps in *Anax* correspond exactly to those of *Æschna*, except that they are elongated in a direction *at right angles* to the longitudinal axis (those of *Æschna* are elongated at a somewhat less angle). The relationships of the rows of leaves in consecutive hemibranchs are the same as those already explained in *Æschna*. Fat-globules and dark pigmentation are present as in *Æschna*, the pigmentation being even deeper than in *Æschna*, and of a purplish-black colour.

Structure of the Papillæ.—The most striking feature in the gills of *Anax* is the very remarkable formation of small papillæ all over the free surface of each leaf or “hump,” which is called on that account by Ris the “basal hump” (die basalen Höcker)—a very appropriate term, if we remember that the hump is “basal” to the papillæ, but “distal” to the efferent trachea forming the axis of the stalk. Oustalet studied these papillæ very carefully, and gives an accurate description and figure of them. They arise in large numbers as small finger-like eversion of the wall of the whole free surface of the hump. They are not homologous with the papillæ already described in *Hemigomphus*, but have been, like these latter, called forth quite independently, in the process of evolution, by the same necessity for obtaining a better extraction of oxygen from the water in the rectum.

Each papilla is of cylindrical shape, with a rounded tip. In proportion, they are shorter and thicker than the papillæ of *Hemigomphus*. Within the papilla *there is no axial trachea*, but only a small number of fine capillary loops, which have been, as it were, drawn or sucked up into the papillar eversion, out of their original course along the surface of the hump, so as to complete their loopings within the papilla. Oustalet estimated that each hump carries a hundred of these papillæ. The number of capillary loops in each separate papilla of *Anax papuensis* varies from five to eight, the average being six (text-fig. 10).

In the cuticle of these papillæ there are no transverse chitinous rods such as are to be seen in those of *Hemigomphus*. At their rounded ends, however, there are a number of little curved chitinous hooks, which probably serve the same purpose as the rods.

Owing to the comparative thickness of the basal humps and the density of their pigmentation, they are even more unsatisfactory for photography than are the foliæ of *Æschna*. Plate 19, fig. 8 shows a profile view of one hemibranch of a half-grown larva, while fig. 7 of the same Plate shows the front

view of parts of two hemibranchs from a somewhat younger larva (7th instar). The drawing in text-fig. 9 is taken from a photograph of the everted gill-basket of a full-grown larva, part of which is reproduced in Plate 21. fig. 19.

Number of Capillary Loops in the Gill-basket.

For the larva studied by him (probably *Anax junius*) Oustalet calculated the number of capillaries at 120,000, allowing only 5 loops to each papilla, 100 papillæ to each hump, and 20 humps in a hemibranch. In *Anax papuensis* there are only 12 humps in a hemibranch, but the average number of loops in each papilla appears to be six rather than five. Hence the approximate total for *A. papuensis* is $6 \times 100 \times 12 \times 12$, or 86,400 complete loops. Oustalet's larva was larger than the one studied by me—hence his total may be well within the mark for that species. *Anax papuensis* is one of the smallest species of the genus, so that we cannot be wrong in stating the average number of loops in larvæ of this genus at over 100,000. Probably an investigation of the larva of one of the largest species, such as *A. gibbosulus*, would yield a very much higher total.

Origin of the Papillo-foliate Sub-type.

It is difficult to indicate precisely the reason for the cænogenetic development of the papillæ on a form of gill already so highly specialized as the *Foliate Type*. In studying the larvæ of *Anax papuensis* and *Æschna brevistyla*, it seemed to me that the reason might be sought for primarily in the difference of habitat. The *Æschna* larva lives in slowly-running water, while that of *Anax* inhabits stagnant pools. Hence it seemed probable that the poorer aëration of the water breathed by the *Anax* larva might have called forth, in the process of evolution, a correspondingly more efficacious type of gill. An objection to this view is that the larvæ of those Palæartic species of *Æschna* which inhabit stagnant water have not developed papillæ. But it seems probable that the larvæ of the genus *Æschna* all originally inhabited running water. Hence, if the habit of breeding in still water, in the case of these Palæartic species, is of very recent requirement compared with the same habit in *Anax* larvæ, my supposition may well be correct.

Dr. Ris has suggested to me (*in litteris*) that the papillæ are correlated with *quickness* of development. The larvæ of all species of *Anax* reach maturity in one year, while those of *Æschna* nearly always take two or three years. Dr. Ris thinks that the rapid growth of the *Anax* larvæ may be principally due to the increased oxygenating power of the respiratory system, due to the presence of the papillæ. This explanation seems to me to be a good one. But it cannot account entirely for the difference in speed of growth. For, as will be shown later (p. 179), the newly-hatched larvæ of

both *Anax* and *Æschna* have gills of exactly similar structure; and, in *Anax*, the papillæ do not begin to develop until the sixth instar. Yet the young larva of *Anax* grows very much more rapidly than the young larva of *Æschna*.

It seems, therefore, that other factors must also play a part in the difference between the two larvæ. I think that one of these two factors is the earlier appearance of the imagines of *Anax*, and the consequent hatching of the larva at a time when the organic life of the ponds is at a maximum. The imagines of the various species of *Æschna* almost all appear late in the summer. Hence the larvæ must hatch out in the autumn, or possibly not until the following spring. Another factor which must play some part is the more sluggish character of the *Æschna* larvæ. I have always found the larvæ of *Anax papuensis*, in my aquaria, to be far more greedy and rapacious than any other Odonate larvæ known to me. The larva of *Æschna brevistyla*, on the other hand, though rapacious enough compared with other Odonate larvæ, cannot be compared with that of *Anax*, either in its alertness in watching for prey or in its capacity for taking food.

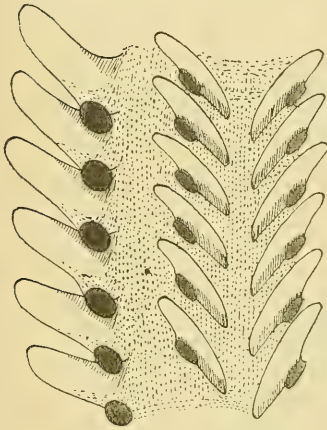
It would seem then that all four causes mentioned above may play a part in differentiating these larvæ one from the other.

3. **Lamellate Type.** (Text-figs. 11-14; Plate 20. figs. 9-12; Plate 21. figs. 13, 14, 20, 21; Plate 22. figs. 24, 27.)

This very distinct and beautiful type of gill-structure is found throughout the whole family of the Libellulidæ. I have studied it in the genera *Synthemis*, *Metathemis*, *Austrocordulia*, *Hemicordulia*, *Cordulephya*, *Orthetrum*, and *Diplacodes*. Sadones's investigations were carried out entirely on the single species *Libellula depressa*, while Ris has studied the gills of European species of the genera *Cordulia*, *Orthetrum*, *Libellula*, and *Sympetrum*. Though, of course, many minor variations in structure are bound to occur throughout so large a mass of forms, yet there are no outstanding differences which seem to me to warrant the erection of sub-types. I propose, therefore, first of all to deal with the general characteristics common to all Lamellate Types of gill, and then to describe the more noticeable differences that appear under the various generic headings.

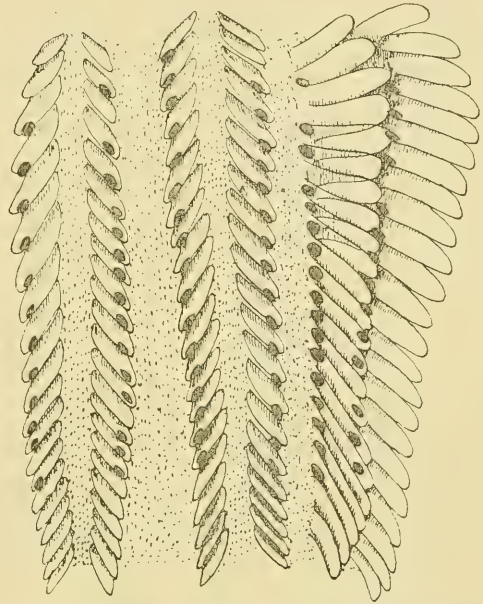
When a lamellate gill-basket is everted by a longitudinal cut, it becomes thrown into a very strongly convex form (the internal portion forming the convexity) so as to resemble a Chinese paper lantern partly opened. On examining this with a low power, six double rows of very regularly arranged flat leaf-like gills or lamellæ are seen projecting from its surface. This very neat and orderly arrangement is in very strong contrast to that exhibited by any other type of gill-basket, and points to the lamellate type as the most perfect form of gill-basket yet evolved.

The general arrangement of the gills is easily understood from text-figs. 11, 12. Each holobranch consists of two exactly similar hemibranchs, symmetrically arranged about a middle line or axis running midway between them. Each hemibranch consists of a single row of gill-lamellæ, ranging in number from twelve to nearly thirty, according to the genus selected. The bases of insertion of these lamellæ are arranged slantwise to the long axis of each holobranch, with their anterior ends close to that axis and their



TEXT-FIG. 11.—*Synthemis macrostigma*, Selys.

Portions of three hemibranchs from the everted gill-basket of a full-grown larva. *Lamellate Type*. The middle and left-hand hemibranchs belong to one holobranch. The right-hand hemibranch is seen in profile from its outer side. Basal pads shown in black. Semi-diagrammatic, drawn from a whole mount. ($\times 30$.)



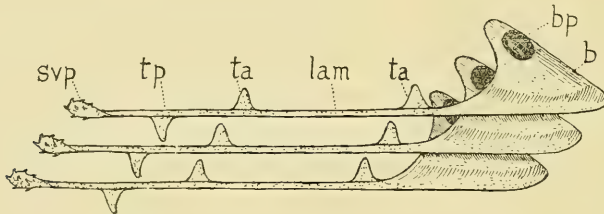
TEXT-FIG. 12.—*Diplacodes hamatodes*, Burm.

One-half of the complete gill-basket, everted, from a full-grown larva. *Lamellate Type*. Pigmentation omitted. Basal pads shown in black, mostly seen by transparency through the overlying anterior lamella. Right-hand holobranchs in profile. Semi-diagrammatic, drawn from a whole mount. ($\times 30$.)

posterior ends removed from it. Hence the gills in each holobranch appear to slant away posteriad from the middle line in symmetrical pairs, one on either side of it. It is quite clear, from a comparison of this figure with that of the Undulate Type in the Simplex System, that these gill-lamellæ represent highly-differentiated cross-folds of an originally Simplex System—for they agree exactly with them both in position and direction. The base-lines of the gills of one hemibranch are, as a matter of fact, intermediate in level

between those of the opposite hemibranch, just as one set of cross-folds is intermediate in position to the other.

The base of each lamella is very broad. From it the lamella itself rises up to project into the rectal cavity in a slantwise direction, with the distal end lying towards the posterior end of the gill-basket. The angle between the rectal wall and the gill-lamella is not constant, but varies with the act of respiration. Also the broad basal part of the lamella is not in the plane of the "blade" or main portion, but curves away from it so as to meet the rectal wall, along its base-line, in a gentle incline (text-fig. 13). Thus, if the lamella be viewed edge-on, the basal portion can be seen rising up above the line of the blade, with the prominent basal pad (*bp*) lying upon it. When the rectum is expanded, the gills stand up in serried ranks, nearly normal to the rectal wall. When the rectum is contracted, the lamellæ fall down so as to overlap one another, all pointing slantwise towards the posterior end of the basket.



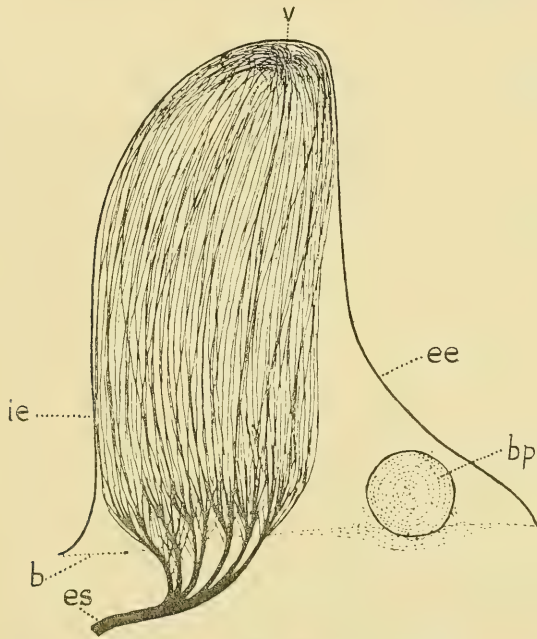
TEXT-FIG. 13.—*Austrocordulia refracta*, Till.

Three lamellæ viewed edge-on to show arrangement of tubercles. Semi-diagrammatic from a camera-lucida drawing. ($\times 90$.) *b*, base of insertion of lamella; *bp*, basal pad; *lam*, blade of lamella; *svp*, supravortical papilla; *ta*, tubercles on anterior face of lamella; *tp*, tubercle on posterior face.

The lamellæ do not, however, lie freely upon one another. They are separated, each from each, by three small tubercles (text-fig. 13) first discovered and described by Sadones in *Libellula*. Apart from these tubercles, the cuticle of the lamella is in most cases perfectly smooth. The tubercles are situated as follows:—One (*tp*) lies near the tip of the lamella on its posterior or lower face; a second (*ta*) lies somewhat distally from the middle on its anterior or upper face; and a third (*ta*) lies close to the base, also on the anterior or upper face. By means of this arrangement, each lamella is separated from the one below it by a *tripod* of three tubercles, of which the outermost belongs to the upper lamella, the two others to the lower. Thus, in all positions of the lamellæ, a constant flow of water can circulate between and around each separate lamella. The advantages of this arrangement for the extraction of oxygen by the gills are so obvious as to need no special comment. In *Austrocordulia refracta* (text-fig. 13) the

tubercles are more prominent and sharply pointed than in most Libellulid genera, and are very clearly seen in profile under a low power.

Basal Pads.—One of the most striking differences between the Lamellate Type of gill and all other forms is the presence of large basal pads belonging to each lamella. These are easily to be seen under a very low power (in *Synthemis* with the naked eye), because of their deep semi-transparent brownish or orange-brown colour. They were first discovered and described by Sadones. If a complete everted gill-basket be examined, it will be seen



TEXT-FIG. 14.—*Hemicordulia tau*, Selys.

A single lamella from a full-grown larva, viewed from the anterior or upper surface. Camera-lucida drawing. ($\times 80$.) *b*, base of insertion of lamella; *bp*, basal pad; *ee*, external edge of lamella; *es*, secondary efferent trachea; *ie*, internal edge of lamella; *v*, vortex of capillaries.

that one of these basal pads is attached to each of the gill-lamellæ, except the first one or two and the last four or five in each hemibranch. (Usually also one can find one or two lamellæ near the middle, here and there, which lack them also.)

The basal pads are a series of rounded disc-like swellings formed in the rectal epithelium at the base of each lamella. They lie towards the outer ends of the lamellæ on the anterior or upper border.

The complete study of these interesting organs, which, although not visible as separate entities in any except the lamellate type of gill, occur in

all forms of gill-basket, is the subject of a special section of this paper (pp. 170-177).

Shape of the Lamellæ.—Text-fig. 14 gives us a very good idea of the general shape of a typical lamella. The base is very broad, and the lamella rises up very unsymmetrically from it. From the outer or distal end, towards which the basal pad lies, the edge of the lamella rises up at a gentle slant, which gradually increases towards the tip. But from the inner end the lamella rises up much more sharply, forming a more regular oval curve. The tip of the lamella may be evenly rounded, or somewhat “nodding” towards the outer side.

Tracheal Supply.—The distribution of primary and secondary efferent tracheæ to the gills is typically that described already for the *Duplex System*, and need not be repeated here (see Plate 22. fig. 27). The tracheal supply of the lamellæ is, however, of great interest. Each lamella receives, beneath its base, one large secondary efferent trachea, which breaks up at once into a number of branches *arising close together*, nearly, but not quite, all at the same level. From these branches there arise whole bundles or pencils of tracheal capillaries, all again very nearly at the same level, close to and just within the base of the lamella. Thus practically the whole of the lamellar area contains capillaries only. The advantages of this arrangement also, for the purpose of the extraction of oxygen, is so obvious as to need no comment.

The capillaries run all very nearly parallel along the lamella until they reach its distal end, where they all loop over inwards to return more along the middle of the lamella, finally entering other bundles which join up to form branches of the secondary efferent below the gill-base. This looping of nearly all the capillaries inwards gives rise, in most cases, to a very characteristic appearance of the capillaries just under the rounded distal end of the lamella, an appearance which I propose to call the *lamellar vortex*. It is most clearly seen in some genera of *Corduliinæ* (text-figs. 14, 15). The in-turning of so many capillaries near the middle line of the gill gives a very distinct appearance of a *vortex* of capillaries at this point. In those genera of *Libellulinæ* in which the tip of the lamella is very regular and rather flat, this vortex is not at all noticeable (Plate 21. fig. 13).

I now propose to take the various genera in phylogenetic order, and indicate the principal peculiarities in the gill-basket of each.

(1) *Synthemis* and *Metathemis*.—The form of the gill-basket is almost exactly the same in the larvæ of *S. eustalacta*, *S. macrostigma*, and *M. guttata*. This form may therefore be considered to be typical of the archaic tribe *Synthemini*. The gill-basket is very small for the size of the larva, and is found on eversion to be of a pure shining white colour without any trace of pigmentation whatever. In each hemibranch there are only *twelve* lamellæ, as against a number varying from twenty to thirty in all other genera of *Libellulidæ*.

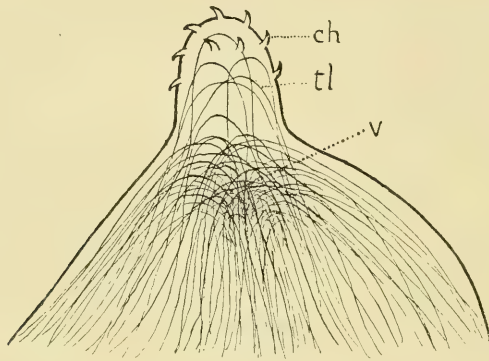
Another striking peculiarity of this gill-basket is the enormous size of the basal pads (text-fig. 11). These appear like rows of large brown buttons along the sides of the lamellæ, and are easily seen with the naked eye. These organs certainly reach their greatest development in this tribe. Their large size indicates probably, however, an earlier stage in the *localization* of these organs as appurtenances of the separate lamellæ. Originally, in the Simplex System, they spread along the whole anterior border of the cross-folds, besides supporting the main folds. When these latter deteriorated, and the cross-folds began to develop as lamellæ, localization of the basal pad began in the shape of a condensation to a rounded pad in a fixed position, with corresponding deepening of pigmentation. Hence the enormous pads of the *Synthemini* may be rightly regarded as an intermediate stage between the non-localized pads of the Simplex System and the extremely reduced separate localized pads of the higher Lamellate Types.

The shape of the lamellæ in the *Synthemini* is fairly typical of the sub-family *Corduliinæ* as a whole, as well as of the lower *Libellulinæ*. Each lamella is distinctly "hump-backed" or "nodding," the inner edge being much more convex than the outer, so that the lamellæ "nod" away from the axis of the holobranch, slantwise towards the posterior end of the rectum. Owing to the narrowness of the gill-basket, the lamellæ of the *Synthemini* are not so elongated as those of other genera. This is particularly noticeable in *Metathemis guttata*, where the breadth of the lamella near its base is nearly as great as the length from base to tip (Plate 20. fig. 9). A distinct tracheal vertex is always present. The capillaries, though very numerous, fall behind those of other genera in fineness.

(2) *Austrocordulia*.—The lamellæ of the gill-basket in the larva of this genus, the only one known for the tribe *Idocorduliini*, is the most remarkable in the whole range of the Lamellate Type. There are a very large number of lamellæ (26 to 30) in each hemibranch, and they are closely packed together. The tubercles separating the lamellæ are large, and easily seen under a low power. The basal pads are fairly large, showing a very dark brown circumference with a pale orange-brown centre. The lamellæ themselves are of typical Corduline form, strongly nodding, considerably longer than wide, and carry a very large number of capillaries of moderate fineness. But the most extraordinary thing about them is that each lamella is furnished, at its nodding tip, *with a short and well-developed papilla*. This papilla I propose to call the *supravortical papilla*, since it lies directly over the lamellar vortex (text-fig. 15). The papilla is a simple eversion of the wall of the lamella at its tip, into which a very few capillary loops have been drawn or sucked. Its structure is essentially similar to that of the papillæ in *Anax*, from which it differs only in being much shorter and wider. Its cuticle is armed with nine or ten strongly-hooked chitinous teeth, all bent towards the outer edge of the lamella. Text-fig. 15 shows this papilla highly magnified.

This papilla also seems to me to be homologous in structure with the tubercles already mentioned. The papillæ in *Anax* arise as small tubercles into which, later on, capillaries become sucked or drawn in. In the case of the tubercles, the swelling up the wall only affects the local epithelium to such an extent as to cause a swelling or thickening of it in the region of the tubercle. By further eversion, the epithelium, and later on the capillaries running in it, could be so drawn out as to bound a hollow cylindrical process—that is to say, a typical respiratory papilla.

The lamellæ in *Austrocordulia* are delicately tinted with a pale mauve or purplish pigment.



TEXT-FIG. 15.—*Austrocordulia refracta*, Till.

Tip of lamella, with supravortical papilla. From a photomicrograph. ($\times 420$.) *ch*, chitinous hooks on papilla; *tl*, loops of capillaries within the papilla; *v*, vortex of capillaries.

(3) *Hemicordulia* (text-fig. 14; Plate 20. fig. 12).—The form of the lamellæ in this genus, representing the tribe *Eucorduliini*, is very typical of that tribe. Each lamella is about twice as long as its breadth across the middle; the actual base-line, however, being much wider, owing to a long slender extension of the distal edge of the lamella. The tip is almost right-angled, the distal or outer edge being nearly straight in this region, while the inner edge is broadly convex. The lamellar vortex is very close to the tip, and only moderately well formed. The number of lamellæ in a hemibranch is usually 24, sometimes 25 or 26. The basal pads are small rounded discs, of a transparent orange-colour. The whole of the gill-apparatus is suffused with the most beautiful delicate purplish-mauve pigment. The capillaries are numerous and of considerable fineness.

(4) *Cordulephya* (Plate 22. figs. 24, 27).—The gill-basket of this interesting genus very closely resembles that of *Hemicordulia*. The lamellæ, however, are slightly longer and narrower, and more regularly rounded at the tips. They resemble the lamellæ of the more typical *Libellulinae* more

than those of any other Corduline genus known to me. There are 22–24 lamellæ in a hemibranch. Delicate purplish-mauve pigment is present as in *Hemicordulia*. There can be no doubt that the form of the gill-basket in this genus is further definite evidence in favour of its being a highly specialized offshoot of the *Eucorduliini*.

(5) *Orthetrum* (Plate 21. figs. 13, 14).—In this genus we see the lamellæ taking on a somewhat more symmetrical shape than is to be noticed in the *Corduliinæ*. This tendency towards symmetry in the form of the upper part of the lamella seems, indeed, to be typical of the *Libellulinæ*. The lamellar vortex is absent, the tips are wide, well rounded, and rather flat, so that the bent or “nodding” effect is almost wholly lost. The lamellæ lie very close together, so that, when a portion of a hemibranch is spread out on a slide, each lamella overlaps not only the one next to it posteriorly, but also a portion of a second one as well. The general effect is one of greatly increased regularity as compared with the type of lamella seen in the *Corduliinæ*.

In *Orthetrum caledonicum* there are 24–26 lamellæ in a hemibranch. The whole gill-basket is of a pure shining white colour, without any trace of pigmentation.

(5) *Diplacodes* (text-fig. 12 ; Plate 21. fig. 21).—This genus shows the most advanced development of the Libelluline type of lamella known to me. The general shape of the lamellæ, which number from 26 to 30 in a hemibranch, is the same as in *Orthetrum* ; but, if anything, the symmetry of the tip is even more marked. A further advance on *Orthetrum* is that the whole gill-basket is so closely pigmented as to appear almost jet-black when cut open. When examined under a low power this pigment is seen to be of a deep purple colour, and is much denser at the bases of the lamellæ than towards their tips. In both *Diplacodes* and *Orthetrum* the capillaries are excessively fine and numerous.

Reviewing the above variations of structure in the Lamellate Type of gill-basket, we see that there is a progressive development from the lower to the higher forms, from which *Austrocordulia* stands out as a separate side-branch, in which an attempt is made to reproduce the papillæ so successfully developed in *Anax*. In some lamellæ taken from a larva of *Hemicordulia tau*, I have noticed the beginnings of a similar attempt at papilla-formation. It seems, therefore, unnecessary to name any sub-types within the Lamellate Type—especially as, when the number of known forms is increased, we may expect to find further intermediate links in the evolutionary chain.

It remains true, nevertheless, that the gill-basket of the *Synthemini* is at once distinguished from all the rest by the size of its basal pads and the small number of lamellæ in a hemibranch, while, amongst the rest, the gill-baskets of the *Libellulinæ* exhibit a general difference from those of the *Corduliinæ* by the greater symmetry of form and the closeness of arrangement of their lamellæ. Hence, if at any time it is desired to subdivide the

Lamellate Type into sub-types, we might propose the term *Archilamellate* for the sub-type exhibited by the *Synthemini*, as contrasted with the *Neolamellate* sub-type found in the other genera. Within the *Neolamellate* sub-type we might then recognize two forms of lamellæ, viz., the *Corduline* form, the more asymmetrical, and the *Libelluline* form, the more symmetrical.

Number of Capillaries in the Gill-basket.—It will be sufficient to indicate the approximate number in the five genera above studied. In *Synthemis* the capillaries are only of moderate fineness, and may be reckoned at only 100 to each lamella. Hence we get a total of $12 \times 12 \times 100$, or only 14,400 capillary loops in this gill-basket. In *Austrocordulia*, there are probably 150 or more capillaries in each lamella. Hence the total for this genus will be $28 \times 12 \times 150$, or 50,400, allowing 28 as the average number of lamellæ in a hemibranch. In *Hemicordulia* the greater fineness of the capillaries may be represented by reckoning 200 in each lamella. Thus the total for the genus is $25 \times 12 \times 200$, or 60,000. In *Cordulephya* the narrowness of the lamellæ allows us only to reckon 150 capillaries in each, in spite of their fineness. Counting, then, 23 lamellæ to the hemibranch on the average, we have a total of $23 \times 12 \times 150$, or 41,400. In *Orthetrum*, again, we can reckon on at least 200 capillaries in each lamella, with an average of 25 lamellæ to the hemibranch. This gives us $25 \times 12 \times 200$, or 60,000, the same as in *Hemicordulia*. The greatest total is given by *Diplacodes* with an average of 28 lamellæ to the hemibranch, yielding a total of $28 \times 12 \times 200$, or 67,200 capillary loops. This number, though it falls short of that calculated for the Foliate Type, belongs, it must be remembered, to a much smaller larva, besides which, the arrangement of the gills in the Lamellate Type is such as to give unrivalled advantage in the extraction of oxygen. We need not be surprised therefore at the fact that the larvæ of *Diplacodes*, *Cordulephya*, and other Libellulid genera develop very rapidly, and pass into the imaginal state well within a year from the time of hatching. What we may well wonder at, however, is the fact that the larva of *Austrocordulia*, in spite of its highly developed gill-basket, takes four or five years to mature. This points, indeed, to some special disability not connected with the oxygen-supply. That disability is, I think, the sluggish habit of the larva, which hides away under rocks or débris, and feeds but seldom.

Comparison of the Efficiency of the Gill-basket in the various Types.

The numbers given as the totals for the capillary loops are in all cases only approximate, and are intended to give an idea of the comparative numerical abundance of capillaries rather than their actual numbers. If we look upon each capillary as a unit for the extraction of oxygen, they enable us to compare the relative values of the different types of gill-basket as oxygen-extractors. It must not be forgotten, however, that the value of the unit also depends upon the length of it exposed to the direct action

of respiration. In this respect the Undulate Type is distinctly at the greatest disadvantage, the Lamellate Type, on the other hand, easily the most advantageous. Forms in which papillæ are developed clearly have an advantage over similar forms without papillæ; but it is doubtful whether a single folia of *Anax*, with all its papillæ, is of any greater value in extracting oxygen than is a single gill of the Lamellate Type.

The following table gives a comparative summary of the number of capillary loops calculated for the different genera examined. The totals are only approximate, but the degree of error in each is probably very much of the same order:—

Species.	System.	Type.	Approximate total.
<i>Austrogomphus ochraceus</i>	Simplex.	Undulate.	10,800 *
<i>Petalura gigantea</i>	"	"	20,000
<i>Hemigomphus heteroclītus</i>	"	Papillate.	18,000
<i>Austroæschna multipunctata</i>	Duplex.	Implicate.	28,800
<i>Dendroæschna conspersa</i>	"	"	46,080
<i>Æschna brevistyla</i>	"	Foliate.	76,800
<i>Anax papuensis</i>	"	"	86,400 †
<i>Synthemis macrostigma</i>	"	Lamellate.	14,400
<i>Austrocordulia refracta</i>	"	"	50,400
<i>Hemicordulia tau</i>	"	"	60,000
<i>Cordulephya pygmæa</i>	"	"	41,400
<i>Orthetrum caledonicum</i>	"	"	60,000
<i>Diplacodes hæmatodes</i>	"	"	67,200

Origin of the Lamellate Type.—It is a matter of great regret to me that, during the year in which this research has been carried out, I have been unable to obtain any Libellulid ova for hatching out the young larvæ. The smallest larvæ that I have been able to obtain were of the genus *Diplacodes*, and measured from 2 to 3 mm. in total length of body. I consider these larvæ to belong to the third or fourth instar. Several of these were sectioned. The results showed, not only that the *Lamellate Type* of gill was already fully established, but that basal pads were already fairly well formed. In no case was I able to discover any sign of the main longitudinal gill-folds of the *Simplex System*, which are the principal breathing-organs in the young larvæ of *Æschna* and *Anax* (p. 143). It is, therefore, clear that the *Lamellate Type*, though necessarily classed within the *Duplex System* because of its structure, has no close phyletic connection with those other types of the

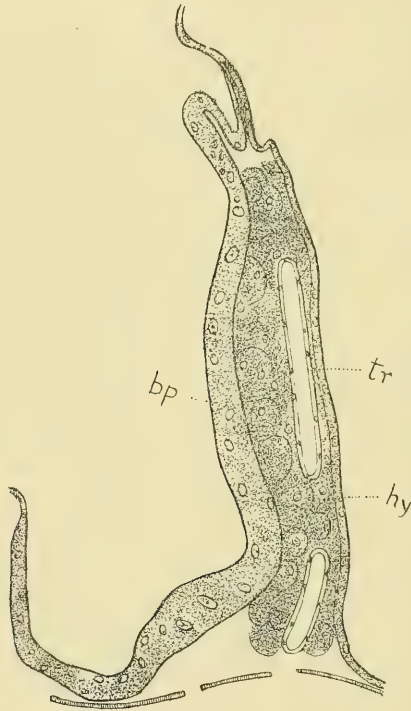
* Minimum.

† Maximum.

Duplex System which we find in the subfamily *Æschninae*. The point cannot be definitely settled, of course, without a careful study of the gill-basket of some newly-hatched Libellulid larva. This I hope to carry out shortly. Meanwhile, we must be content to regard the *Lamellate Type* as arising from a very simple and early form of the *Simplex System* by the complete suppression of the main folds, and the development of the cross-folds into lamellæ.

THE BASAL PADS. (Text-fig. 16 ; Plate 22. figs. 25-27.)

We owe to Sadones a very excellent and careful study of these interesting organs in *Libellula depressa*. In this species—and, in fact, in all the Libellulidæ—the formation of the basal pads is very highly specialized. Clearly, then, it would be both unwise and unprofitable to speculate on the origin of such a highly developed structure without at first studying its formation in the less



TEXT-FIG. 16.—*Dendroæschna conspersa*, Till.

Transverse section through base of gill from a full-grown larva. ($\times 440$.)

bp, basal pad ; *hy*, hypobranchial tissue ; *tr*, trachea.

highly specialized form of gill-basket. In attempting to indicate the boundaries of each cell-territory of this peculiar structure, it is probable that Sadones was somewhat misled by his lack of knowledge of the structure

of this organ in other forms besides *Libellula* ; for, as we shall show, the basal pad was first formed *much later than* the establishment of the syncytial nature of the rectal epithelium, and hence must be regarded as *never having possessed separate cell-territories at all*.

Looking through our series of gill-forms, we are at once struck with the fact that it is not in the *Simplex System*, which is phylogenetically the most archaic, that the simplest form of basal pad is found. This is, perhaps, explicable on the supposition that the more isolated positions of the gill-folds in the *Simplex System* render the support of basal pads more necessary than is the case with the *Duplex System*. In any case, it is a fact that well-developed basal pads occur in all forms except the *Implicate Type* of the *Duplex System*. We shall therefore select, first of all, for study the basal pads, if such they may be called, of *Dendroaeschna conspersa* (text-fig. 16, *bp*). It is only necessary to recall, in attempting to explain the rudimentary nature of these structures, the fact that in the *Implicate Type* of gill the separate grooved folds lie alternately with their rounded ends supported along a wavy central line, so that there is actually no complete separation of the separate portions of the holobranch into foliæ or lamellæ. Hence the holobranch is more or less self-supporting, and a well-developed basal pad is not wanted.

In *Dendroaeschna* the base of the gill-fold is only very slightly enlarged to receive a narrow elongated strip of hypobranchial tissue (*hy*), in which the gill-trachea (*tr*) runs so straight and regularly that it is very often met with from end to end in the same cross-section. Our figure shows it cut in two places. On the inner side of the gill-fold, the ordinary flat syncytial epithelium borders the hypobranchial tissue along its whole length, and passes round along an arc of the rectal circumference for a short distance, to rise again towards the interior on the internal side of the next gill-fold. But, in sections which show the efferent trachea, the *external* epithelium of the base of the gill-fold is slightly swollen into a rudimentary basal pad (*bp*). The protoplasm of this pad shows, so far as I have been able to study it, no trace of any fibrillar structure. It is, in fact, similar to the protoplasm of the epithelial syncytium in its ordinary flat condition, except that it stains slightly darker with hæmatoxylin. Towards the two ends of the pad the nuclei are rather numerous, but not different in size or structure from those met with in other parts of the syncytium. But, in the main portion of the pad, the nuclei are very distinctly enlarged, mostly very distinctly oval in form, and show granular contents, and in many cases a fairly distinct nucleolus. On the whole, these nuclei stain less deeply than those of the syncytium, and their contents are more clearly differentiated.

Passing away from the level of the efferent trachea, the sections show a rapid decrease in the width of the basal pad, which very soon merges into the typical epithelial syncytium of the gill-fold. These pads, therefore,

appear to be in the nature of rudimentary supports developed in conjunction with the main efferent tracheæ and their accompanying hypobranchial tissue. But, uninteresting as the structure of the basal pad in *Dendroæschna* may be, it nevertheless allows us to make certain very obvious deductions. The first is that it is almost certain, with this example before us, that all basal pads arose merely as swellings in an originally uniform epithelial syncytium. The second is that they were probably called forth to act as supports for the bases of the projecting gills, and play no part in the physiological process of the extraction of oxygen from the water in the gill-basket. The third deduction is that the basal pads *never possessed separate cell-territories*, and therefore that it is useless to try, as Sadones has done, to indicate such territories around each nucleus.

I should like here to digress in order to institute a comparison between the basal pads of the gill-basket and the well-known fibrillar columnar epithelium which supports the "fields" bearing the teeth in the gizzard of Odonate larvæ. There also the need is for a support, and the need is supplied by the specialization of the epithelium underlying the chitinous cuticle. In the gizzard, however, the epithelium is formed of separate cells. Hence we find the columnar support evolved from it also formed of separate cells. These cells, however, agree with the syncytium of the basal pad in having their nuclei enlarged, and in showing a distinct fibrillar structure and a turgescient appearance. The function of these enlarged areas, whether in the gizzard or in the gill-basket, is clearly that of a cushion or support for a structure which might otherwise collapse.

To return to the basal pad. Apart from those of the *Implicate Type* in the *Duplex System*, the basal pads of all forms examined by me show a high degree of specialization. Let us take the pad found in *Austrogomphus ochraceus* (Plate 22. fig. 22). At the bases of both main longitudinal folds and cross-folds, lying on the outer side of the gill-wall, one finds these very prominent pads in all sections. They lie not only along the projecting gill-fold itself, but also partly along a small portion of the circumference of the rectum. In many sections the pad may be seen to be continuous between a main fold and an adjacent cross-fold. In the enlarged figure of a basal pad selected from the section diagrammatized in Plate 22. fig. 22 (fig. 25 of the same Plate), representing the pad marked * in the diagram, the pad will be seen to rear itself high up on one side against the wall of the main fold, while another and shorter portion bends upwards against the base of the cross-fold. Between, a portion of the pad lies on the rectal circumference. If this pad be followed through a number of sections, it will be seen that the part supporting the base of the cross-fold increases as the cross-fold comes to be cut more centrally; afterwards it decreases as the section of the cross-fold decreases.

The pad is continuous at both ends with the epithelial syncytium, and

forms a highly specialized portion of it. Its protoplasm is only very slightly chromatophil, clear, very slightly granular, and having embedded in it a series of comparatively enormous nuclei (nu_1), besides a smaller number of much smaller nuclei (nu_2). There is absolutely no indication of separate cell-territories in this mass. On its external side it is covered by a continuation of the fine cuticle which everywhere overlies the rectal epithelium. On its inner side, it is contained by a very delicate basement-membrane separating it from the adjacent mass of hypobranchial tissue.

The most noticeable feature in the structure of this pad is the abundant fibrils which cross it transversely from side to side. Many of these fibrils are seen to be attached to the nuclei, and therefore clearly do not represent cell-limits. Wherever the pad is bent or creased, there may be seen particularly strong fibrils arising, sometimes in groups, to pass out divergently across the pad. Generally, a number of fibrils pass out from each nucleus and tend to diverge as they approach the border of the pad. The spaces between the fibrils are clear for the most part, appearing slightly more granular towards the edges of the pad. The whole appearance of these spaces suggests a state of turgidity, and they are very probably filled with a liquid of some sort, the actual protoplasm of the pad being confined to the nuclei, the fibrils, and the granular substance which lies close to the borders of the pad.

The large nuclei, which I propose to term the *meganuclei of the basal pad* (nu_1), are rather unevenly scattered through the pad. In *Austrogomphus*, one finds the greater number of them lying rather closer to the external than to the internal border of the pad. In that part of the pad which lies on the rectal circumference, they tend perhaps to lie even more unevenly, for one not infrequently sees one, or two close together, lying fairly close to the basement-membrane. Throughout the pad, there seems to be a tendency for two or three meganuclei to approximate together; so that, in any given section, one meets with several cut through very close together, forming as it were a centre of radiation for numerous fibrils, and followed by a space in which no nuclei are visible.

These meganuclei are all of a somewhat elongate-ovoid shape, showing in a cross-section of the pad an elliptical section with its major axis transverse to the pad, and in longitudinal section of the pad a circular or nearly circular section. Hence they are, in actual shape, elongate *prolate spheroids*, with their axes directed transversely across the pad at right angles to its internal and external borders. If we suppose the basal pads to have been formed by the absorption of some liquid into the protoplasm of a portion of original typically flat epithelial syncytium, in such a way that the swelling took place everywhere generally at right angles to the plane of the epithelium, both the fibrils and the nuclei would become extended in the direction in which we here find them. It seems then probable that the meganuclei were

the *original nuclei* of the epithelial syncytium, which have become enlarged in a given direction by the gradual growth of the pad perpendicular to the epithelial plane.

The meganuclei are chromatophil, staining fairly deeply with hæmatoxylin in contrast to the surrounding substance of the pad. Each meganucleus contains a very definite and darkly staining nucleolus, together with numerous scattered granules, also very darkly staining.

Here and there in the sections of the pad, but more especially towards its ends, there are to be seen smaller nuclei (nu_2), which I propose to distinguish by the name *micronuclei of the basal pad*. These are nearly always found to lie near the internal border of the pad. They are darkly stained, usually showing a nucleolus and close granular contents. In shape they appear to be flattened or elongated a little in the opposite direction to the axis of the meganuclei; very often they are nearly circular in cross-section. These I also regard as originally nuclei of the epithelial syncytium, which, for some reason or other, have failed to swell out into meganuclei during the development of the pad. The fact that they lie near the ends of the pad partly explains this occurrence. That they also lie close to the internal border of the pad seems to me a strong argument for the development of the pad by imbibition of watery fluid from the rectum; for, if such be the case, those nuclei which lie closest to the external surface would naturally respond first to the access of fluid.

In putting forward the above theory as to the derivation of the basal pad and its nuclei, I may add that, in young larvæ of *Anax* and *Æschna* which I have examined and sectioned from the first to the fourth instars, there are no traces of basal pads. Their development, then,—in these genera, at least,—takes place in later stages. This fact seems to me to make the supposition of their development by imbibition of water from the rectum a very probable one—at any rate, it could not be urged as a solution if we had found the basal pads already developed in the newly-hatched larva.

The basal pads of *Hemigomphus* and *Petalura* are essentially similar in structure to those of *Austrogomphus*. Those of *Petalura* were, however, not so wide in comparison to their length as in the two Gomphine genera. Little weight, however, can be attached to this circumstance, since the larva of *Petalura* which I sectioned had been in alcohol for over four years, and there were very obvious signs of the complete extraction of the watery fluid from the pads, which in some parts had actually collapsed or broken open, showing clear unstained spaces between the fibrils. The complete absence of any substance between the fibrils in the pads of this larva points to the probability of their original contents having been largely of a watery nature, and gives further support to the theory outlined above.

In the three genera under discussion, as well as in the *Æschninae*, I have never seen, on opening the gill-basket, any sign of definite basal pads of a

rounded or elliptical shape and deep orange-brown colour, such as are readily noticeable in the gill-baskets of the Libellulidæ. The pads do not, in fact, show up until the basket has been sectioned and stained. It is clear then that they are widely diffused structures forming a more or less general support to the gill-folds. Judging by their occurrence in a long series of cross-sections, I conclude that they support the main longitudinal folds alternately on either side, sending off side-branches along the *anterior* sides only of the cross-folds. In this connection it is important to notice that, in the Libellulidæ, the localized oval pads occur only on the *anterior* sides of the gill-lamellæ. Also, the form of the basal pad and its nuclei in the *Gomphine* and *Petalura* (apart from its actual size, shape, and position) is very closely similar to that of the Libellulidæ. These two points are of great importance when we come to consider the homologies of the gill-lamellæ in Libellulidæ.

Passing on now to the basal pads found in those *Æschnine* which have gills of the Foliate Type (*Æschna* and *Anax*), we have to notice two very important points of difference between them and those just studied above. Plate 22. fig. 26 shows a very typical cross-section taken from a foliate gill near the posterior end of the gill-basket in *Æschna brevistyla*. The outstanding feature of this section is the *occurrence of basal pads on both anterior and posterior faces of the folia*. Of these, that on the anterior face is the larger and longer, extending through a greater number of sections and showing everywhere a greater length along the gill-base. The second point is the more rounded form of the meganuclei (nu_1). These, though nearly always distinctly oval in transverse sections of the pad, are not of the elongate-oval form seen in the pads of *Austrogomphus*. Correlated with this rounder form of the meganuclei is the greater narrowness of the pad compared with its length, when contrasted with that of *Austrogomphus*. Micronuclei are also present, of the usual size and form.

In *Æschna* no part of the pad lies along the rectal circumference, but the whole mass stands up as a support for the base of the gill. Between the two pads lies a large mass of hypobranchial tissue (*hy*), while a very distinct cavity (*sp*) is often to be seen between the upper ends of the pads. At both ends the pads narrow to pass into the epithelial syncytium, which is *very deeply pigmented* (*pg*).

The last type of basal pad to be studied is that found throughout the Libellulidæ (Plate 22. fig. 27). This type differs from all those studied above in its intense localization into the form of separate oval or rounded swellings situated at the base of each lamella—a localization which is evidently correlated with the separation of the gills into distinct lamellæ.

Sadones, who studied this form in *Libellula*, made a peculiar error in stating the position of the pads on the lamellæ. He states that they lie near the axis of symmetry of each double series of lamellæ. By reference to

text-fig. 12, which is drawn from a photograph of an opened and cleared gill-basket of *Diplacodes hematodes*, and also to text-fig. 11 (*Synthemis*), it will be seen that this is by no means the case. On the contrary, each pad lies at the *outer anterior extremity* of the basal line of insertion of each lamella, and hence it is as far as possible from the axis of symmetry. This can also be easily verified in transverse sections, where the pads are always found lying on the *external* sides of their lamellæ (Plate 22. fig. 27). Now, since the basal line of insertion of each lamella lies in a slanting direction to the longitudinal axis of the gill-basket, with its inner end considerably *more anterior* than its outer, it follows that the external position of the pad in cross-sections indicates an actual position at the far end of the lamella and on its *anterior* side. Sadones's error obviously arose from his failing to distinguish clearly the two sets of lamellæ belonging to one double series. He has, by error, selected two adjacent hemibranchs from left and right respectively of two adjacent double series. Hence his axis of symmetry is really a line midway between two double series, and the position of the basal pads is correspondingly misdescribed.

Plate 22. fig. 27 shows a transverse section through one single series of lamellæ in *Cordulephya pygmæa*, enlarged from a portion of the complete section shown in fig. 24. Apart from their shape, the structure of the pads closely resembles that already described in *Austrogomphus*. The pad is more thickened than in *Æschna*, and the meganuclei are quite as elongated as those of *Austrogomphus*.

Sadones mentions the occurrence, in the basal pads of *Libellula*, of an occasional small nucleus, considerably smaller than the micronuclei, lying close up to the cuticle. I have also noticed similar nuclei in the pads of other Libellulidæ, but I may add that they are of very rare occurrence. One of them is shown in fig. 27, *nu*₅.

The very striking shape and colour of the basal pads in Libellulidæ is worthy of remark. On opening a gill-basket, the pads appear to be rows of buttons, of a rich transparent orange-brown colour, surrounded, in *Austrocordulia*, by a ring of very dark brown. It is somewhat doubtful whether this colour actually belongs to the pad or to the underlying hypobranchial tissue. But, judging by its regularity of shape, I am inclined to attribute it to the pad itself, since the masses of hypobranchial tissue are of far less regular shape, and often project outwards below the bases of the pads.

The shape of the pad appears different according to the point from which it is viewed. When the gill-basket is everted, the pads at either end of a hemibranch are viewed somewhat end-on and appear as very elongate ovals. Towards the middle of the rows the convexity of the basket may throw the pads upward so that a more direct view is obtained. Viewed thus from above, they appear either circular or very convexly triquetral. It will thus

be seen that the pads are actually circular or nearly circular discs of fairly considerable thickness.

In the great majority of Libellulidæ the lamellæ are very numerous, more than twenty in a hemibranch, and very closely set. In these, usually the first two or three and the last four or five will be found to possess no basal pads at all. Occasionally also a pad may be missing here and there on any chance lamella near the middle of a hemibranch.

In the *Synthemini*, the number of lamellæ in a hemibranch is only twelve, or occasionally thirteen, and the lamellæ stand correspondingly further apart. Usually every lamella has a basal pad, but one may be absent here or there, especially on the first one or two lamellæ. The basal pads are comparatively enormous, at least twice as large as in other Libellulidæ (text-fig. 11), and their diameter may be as much as one-fourth of the whole length of the lamella. Their large size seems to be explicable in two ways, viz. :—

(1) Owing to the greater space between consecutive lamellæ, as compared with those of other Libellulidæ, there is a correspondingly greater need of basal supports. This is met by the greater size of the basal pads.

(2) The *Synthemini* are admittedly the most archaic of all Libellulidæ. If, then, we may assume that the localized basal pads of the Libellulidæ are derived from diffuse pads such as are found in gills of the Simplex System, we should naturally expect to find *less* localization in the more archaic *Synthemini*. The large pads of the latter, then, may be regarded as intermediate in formation between the diffuse pads in the Simplex System and the smaller localized pads of the rest of the Libellulidæ. It seems clear that the decrease in size of the pads is correlated with the increase in number of the lamellæ in each hemibranch and their greater closeness to one another.

THE HYPOBRANCHIAL TISSUE. (Text-fig. 16 ; Plate 22. figs. 25–27.)

In all forms of gill the space between the two gill-walls at the base of the gill-fold, bordered on one or both sides by the basal pad, is more or less completely filled with a mass of tissue in which the principal tracheæ of the gill run. This tissue was called by Chun and Faussek "connective tissue." Sadones called it "adipose tissue," but admitted that this name was faulty, since no trace of fat can be found in it. I propose to term it *hypobranchial tissue* (*hy*), a name which avoids any assumption as regards either structure or function, both of which are somewhat problematical, and simply indicates its position as lying in the space at the base of the gill.

This tissue differs from ordinary adipose tissue, not only in the absence of fat, but also in its staining qualities. With hæmatoxylin, it stains almost to an equal intensity with the basal pad ; so that, apart from its more irregular shape and the difference in the shape of its nuclei, it might be difficult to pick out at once in sections treated only with this stain. I find,

however, that this tissue takes a light but very characteristic eosin staining, which distinguishes it very clearly from the basal pad.

The structure of this tissue is the same in all forms examined, though the size and shape of the masses varies greatly. In all cases, definite cell-boundaries are not easily made out. The masses seem rather to consist of rounded lobules, sometimes with only one nucleus, but more often with several in each lobule. Usually the curved borders of the lobules show free and uncompressed, and there is very seldom seen any polygonization of the masses by mutual compression. The nuclei (*nu*₄) are numerous, of moderate size, rounded, darkly staining with hæmatoxylin, and with a fairly large and very distinct nucleolus.

Owing to its lobulate formation, the hypobranchial tissue never completely fills the space at the base of the gill-fold. It is not, however, therefore necessary to assign any particular function to the small space left at the top, just below the union of the two gill-walls. Sadones has termed this cavity a blood-space, and based upon it a theory that the blood plays some part in the physiological processes of respiration. Against this, I am able to state definitely that blood-corpuscles never occur in these cavities, and it seems very doubtful whether they have any regular communication with the hæmocœle at all. There is certainly no regular circulation of fluid in them, nor have I been able to trace in them either any regularity of form or any definite outlets to the hæmocele.

The most definite fact about the function of this tissue is that it carries within its meshes, in all cases, the large trachea or tracheæ which enter the bases of the gill-folds. It is thus a support surrounding these main tracheæ, and I am inclined to regard this as its principal function. If we remember that the gases received from the water in the rectum must already have traversed the capillary loops and smaller tracheal branches before they reach these larger tracheæ, and that, by this time, these gases are simply in process of being transmitted to the main tracheal trunks for distribution throughout the body, we must see that the position of this tissue almost forbids us from ascribing to it any really important part in the physiological processes of respiration.

As to the origin of the hypobranchial tissue, I should like to state my belief that it will be found to be a special development from the supporting tissue to be found in large masses under the raised epithelium of the so-called "rectal glands" in the posterior portion of the rectum. The purview of this paper did not, of course, embrace a study of this portion of the rectum. But, in tracing back the posterior ends of the duplex gill-system in *Æschna brevistyla* (text-fig. 8), I had to follow the gill-basket in section right through into the region of the "rectal glands." Here I was struck by the immense masses of supporting tissue (*st.*) lying under the raised rectal epithelium. It seems probable that this tissue occurs in the recta of all

Odonate larvæ. In fact, I have already seen it in several genera of Zygoptera as well as in *Æschna*. Now we have already shown that the main gill-folds of the *Simplex System* are homologous with the "rectal glands." Their hypobranchial tissue, therefore, corresponds in position with the supporting tissue of the "glands."

The differences between the hypobranchial tissue and the supporting tissue of the glands are obvious enough. The former stains with eosin, the latter does not. After treatment with soap-alcohol, the former is unaffected, the latter has its contents extracted and shows up as a reticulated network of more or less empty spaces, much resembling the "skeleton" of the fat-body obtained by the same reagent. Thus, while the supporting tissue is closely analogous to true adipose tissue, and probably contains considerable stores of fat, the hypobranchial tissue is of a very different nature, and the contents of its lobules are impervious to the action of soap-alcohol. This suggests that the nature of the tissue has become chemically altered by the assumption of some new function connected with respiration, possibly the extraction of nitrogen from the gas passing along the tracheæ. That question, however, cannot be decided here, but must be left for future physiological investigations.

I have figured the hypobranchial tissue (*hy*) in the gills of *Dendroæschna* (text-fig. 16), *Austrogomphus* (Plate 22, fig. 25), *Æschna* (Plate 22, fig. 26), and *Cordulephya* (Plate 22, fig. 27). In *Dendroæschna* it is, like the basal pad, very narrow in cross-section. In *Austrogomphus* it usually fills up the hypobranchial space fairly completely; while in *Æschna*, where its mass is greater and more irregular, it often leaves a considerable space both above and below. In the Libellulidæ it may appear as a single row of lobules (possibly separate cells), or may be more irregular, with two or more layers of lobules in some cross-sections.

ONTOGENY OF THE GILLS IN THE LARVÆ OF *ÆSCHNA* AND *ANAX*. (Text-figs. 17-20.)

The development of the gills in the rectum of the growing larvæ of *Æschna* and *Anax* is a process of very great interest, and supplies the necessary evidence for linking up the structure of the *Simplex* and *Duplex Systems*.

A number of eggs of *Anax papuensis* were obtained by me in February last. Those that hatched out were taken on to the different larval instars as far as the fifth, two or three being killed immediately after ecdysis for the purposes of sectioning. Unfortunately the supply was not large enough to carry the investigation beyond the fifth instar. But the deficiency was here supplied by the discovery of a number of larvæ of *Æschna brevistyla* in very early stages, easily seen to correspond exactly with the fourth and fifth

instars of *Anax*. One of these was raised to the seventh instar. Later, a large number of *Anax* larvæ were found in stages which clearly corresponded to this seventh instar of *Æschna*, besides a few in the sixth instar. Many of these are still alive and at present are in the eighth or ninth instars (August). As the formation of the duplex foliate gill-type is practically completed by the seventh instar, these larvæ supplied me, *in toto*, with a complete account of the gradual formation of the gill-system.

We shall divide our account of the ontogeny of the gills into three sections, as follows:—

- (1) The state of the gill-basket at the time of hatching, and the changes that take place immediately afterwards.
- (2) A description of the gill-basket and its tracheal supply during the second instar*.
- (3) The gradual development of the duplex system of gills.

- (1) *The state of the gill-basket at the time of hatching, and the changes that take place immediately afterwards.* (Text-figs. 17–19.)

The young *Anax* larva emerges from the egg very rapidly, covered with two cuticles. The *first instar*, or *pronymph* stage, lasts only a very short period, ranging from a few seconds to a little over a minute. Then the outer cuticle is rapidly cast off, and the larva emerges fully equipped for its life-struggle, with its powerful mask formed ready for capturing its prey. This is the *second* instar.

At the moment of emergence from the pronymphal skin, the pulsating organ of the head is still beating, though it subsides shortly afterwards. The tracheæ of all the posterior region of the body contain no air and are quite invisible, except the large dorsal trunks, which can be faintly seen. The dorsal tracheæ, in the region anterior to the midgut, are seen to be filled with air, which appears like a black continuous rod travelling steadily backwards along the tracheæ. At the moment when the larva gets free from the pronymphal skin, the air has reached to the level of the midgut. Thence it travels fairly quickly backwards, filling up and outlining in black each tracheal branch which it meets with in its course. I am inclined to connect this regular filling of the tracheæ with air, from some point in the anterior part of the body, with the pulsations of the head organ, which can be seen to stop about the time that the tracheal system becomes filled with air. The point, however, needs further elucidation.

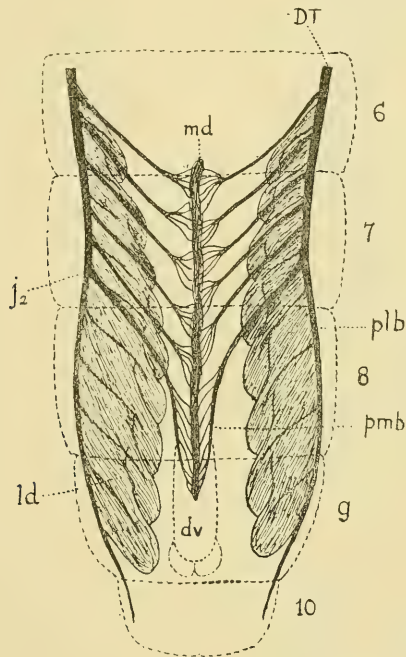
At first, there is no sign of gills in the rectum. But as the air travels backwards to the rectum, six longitudinal gills begin to be outlined in very regular and beautiful fashion. These gills are thrown into folds or undulations in which numerous tracheal loops occur. The air enters each loop in

* Here, as elsewhere, I consider the so-called "pronymph" stage to be definitely the *first instar*, so that the larva commences its free existence in its *second instar*.

turn and travels round it completely, until the whole series of folds with their contained loops is clearly outlined by the air within. Regular movements of contraction and expansion of the rectum take place from this time onward.

(2) *A description of the gill-basket and its tracheal supply during the second instar.*

We have seen that the original air-supply of the gill-basket is not derived from water drawn into the rectum, but comes from some source in the anterior portion of the larva, and enters the gills through the main tracheal trunks. Let us now study the formation of the gill-basket as it is to be seen in the transparent larva shortly after the first ecdysis.



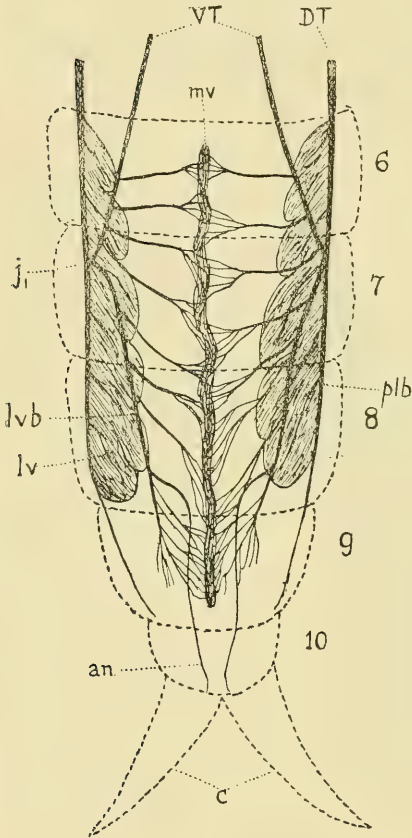
TEXT-FIG. 17.—*Anax papuensis*, Burm.

Dorsal view of gill-basket of newly-hatched larva. Camera-lucida drawing. ($\times 180$)
 DT, dorsal tracheal trunk; *dv*, dorsal vessel or heart; *j₂*, second junction; *ld*, latero-dorsal gill-fold; *md*, mid-dorsal gill-fold; *plb*, postero-lateral branch of DT; *pmb*, postero-median branch of same. 6-10, abdominal segments.

The six longitudinal gill-folds lie in the rectum in positions corresponding to the numbers 2, 4, 6, 8, 10, 12 on the clock-face. They are not all of the same length, the ventral gill-fold and the two latero-dorsals (*i. e.* the three gills corresponding to the numbers 2, 6, 10 on the clock-face) being

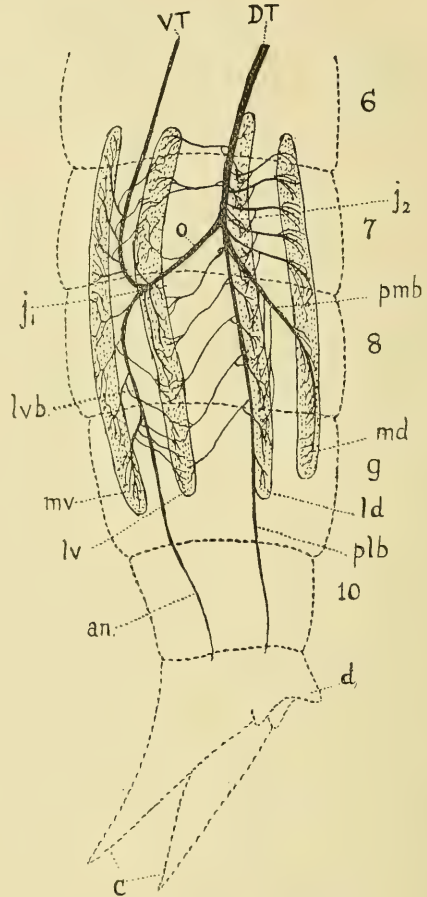
considerably longer than the other three, and the dorsal gill being the shortest of all. The lengths of the gills are not constant, for, during the movements of expansion and contraction of the rectum, not only their actual lengths, but also their positions with respect to the abdominal segments, become slightly altered.

Each gill-fold is thrown into from six to nine very distinct undulations,



TEXT-FIG. 18.—*Anax papuensis*, Burm.
Ventral view of gill-basket of newly hatched larva. Camera-lucida drawing. ($\times 180$.)

an, anal branch trachea ; *c*, cerci ; *j*₁, first junction ; *j*₂, second junction ; *lv*, latero-ventral gill-fold ; *lvb*, latero-ventral branch of DT ; *mv*, mid-ventral gill-fold ; VT, visceral tracheal trunk. Other references as in text-fig. 17.



TEXT-FIG. 19.—*Anax papuensis*, Burm.
Lateral view of gill-basket of newly hatched larva. Camera-lucida drawing. ($\times 180$.)

d, appendix dorsalis ; *o*, oblique trachea.
Other references as in text-figs. 17-18.

in each of which a number of complete capillary loops can be clearly seen. The epithelium of the gills appears to be of a slight yellowish tinge.

The study of the two pairs of lateral gills is not easy, as it is difficult to persuade the larva to rest in suitable positions. However, I was able to make camera-lucida drawings of a larva, not only from the dorsal and ventral aspects (text-figs. 17, 18), but also in profile (text-fig. 19). The actual distribution of tracheæ in the gill-basket was most carefully studied in the case of dorsal and ventral gills, so that I will deal with these first.

From the dorsal aspect (text-fig. 17) the dorsal gill-fold is seen to be a long undulating strand of tissue lying in the middle line. From the main dorsal trunk (DT) on each side, six efferent tracheæ come off slantingly and run towards the gill-fold. Just before reaching it, each efferent trachea divides into several secondary branches. These branches pass into the gill vertically downwards in alternate sets to right and left. Hence arises that slight undulatory outline of the base-line of the gill-fold; for each set of tracheæ may be supposed to exert a very slight pull on the gill towards one side, so that each set enters the gill by a slight convexity on its own side. It will thus be seen that the system of gills present in the larva of *Anax* soon after hatching is very different from that seen in the older larva. It is, in fact, a primitive *Simplex Gill-system* of *Undulate Type*, very similar to that found in the well-grown larvæ of *Petalura*, *Cordulegaster*, and *Austrogomphus*, but without any system of cross-folds. This fact fixes the *Undulate Simplex* type of gill definitely as the *most primitive* of all the types studied in this paper.

The last of the six efferent tracheæ (*pmb*) on each side of the dorsal gill is longer and thicker than the preceding five. Instead of breaking up into a few secondary branches, it turns to run posteriad alongside the gill for some considerable distance, giving off at short intervals some eight or nine secondary branches. Thus these last two efferent tracheæ come to supply between them nearly one-third of the whole length of the gill. By referring to text-fig. 17, it will be seen that these tracheæ, which I have designated the *postero-median branches* (*pmb*) of the main dorsal trunk, correspond exactly with the strong trunks of the same name which supply the narrow posterior portion of the gill-basket in the full-grown larva of *Æschna brevistyla*.

Turning now to the ventral gill-fold (text-fig. 18), we notice a very similar arrangement of efferent tracheæ supplying the gill alternately from left to right. There are, however, some important differences. Corresponding to the greater length of this gill-fold, we find altogether *eight* efferent tracheæ on each side. Of these, the first four on each side arise from the corresponding *visceral trunk* (VT). Just posterior to the fourth, however, the visceral trunk bends round upwards to enter the *oblique branch* (*o*) of the main dorsal trunk at the point which I have called the *first junction* (*j₁*).

Arising also from this junction, a strong trachea, the *latero-ventral branch* (*lvb*), passes posteriad to the region of the anus. From this trachea the remaining four efferents are given off to the ventral gill-fold. The last efferent of all appears to be a straight continuation of *lvb*, and gives off numerous secondary tracheæ to the ventral gill-fold. The anal branch (*an*) comes off from *lvb* at the point where we consider the last efferent to begin, curves inwards towards the middle line, and then passes to the end of the tenth segment.

During the movements of respiration, the oblique branch *o* swings to and fro, so that its angle of inclination to the dorsal trunk constantly varies. Hence the position of the first junction (j_1) is always altering. Sometimes it is seen to lie just under the suture between segments 7 and 8 (text-fig. 19), sometimes it appears in the anterior portion of segment 7 (text-fig. 18). The structure and size of this oblique branch strongly suggests that both the visceral trunk and the latero-ventral branch are true outgrowths of the main dorsal system, and probably receive their air in the first instance via the oblique branch. I was not, however, able to observe this taking place during the very short period in which the air was travelling along the tracheæ.

When we turn to the lateral gills (text-fig. 19) we can see that each gill-fold is supplied by two sets of efferent tracheæ, just as in the case of the dorsal and ventral gill-folds. In the case of the latero-dorsal gill-folds, these sets are both derived from the dorsal trunks, but in different manners. My observations on their distribution are not so complete as I could wish, but I was able to make out the following points. From the point where the large postero-median branch (*pmb*) comes off, the dorsal trunk runs posteriad almost to the anal end of the larva. This point I have termed the *second junction* (j_2). It marks also the branching off of the oblique branch (*o*) from the main dorsal trunk. One set of efferent tracheæ, numbering eight or nine, comes off from the whole length of the main dorsal trunk in this region to supply the dorso-lateral gill-fold, while a second appears to branch off from the efferent tracheæ of the dorsal gill-fold, close to their points of origin. Owing to the great foreshortening of these tracheæ in lateral view, their exact distribution was difficult to make out; while, in dorsal view, the laterally-lying position of the latero-dorsal gill-folds made it impossible to study their points of entry into the gill at all.

With regard to the latero-ventral gill-folds, the same difficulties were met with, but in a less degree, as the amount of foreshortening was less. A set of seven or eight efferent tracheæ enters these gills on their dorsal side, and arises from the dorsal trunk. They seem to be united near their bases with the lower set of efferents to the latero-dorsal gill-folds—that is to say, the dorsal trunk on each side gives origin to a set of tracheæ, each of which branches into two near its base, one branch passing to the latero-dorsal gill-fold, the other to the latero-ventral. The other set of tracheæ supplying

this latter gill arise partly from the visceral trunk and partly from the latero-ventral branch, from the same stems as the efferents of the ventral gill-fold ; they also are seven or eight in number.

It will be seen from the above description that, although the form of the gill-folds is very simple, their tracheal supply is very complicated. It may be best understood by ignoring, for the moment, the complications introduced by the branchings of the tracheal trunks at j_1 and j_2 , and confining our attention to the part of the gill-basket anterior to these two points. We may then state the position as follows :—

Each dorsal trunk gives off alternately *two* sets of primary efferent tracheæ, a more dorsal set and a more ventral set. Each trachea of the more *dorsal* set divides into two branches, one of which becomes a secondary efferent trachea of the dorsal gill-fold, the other a secondary efferent of the latero-dorsal gill-fold. Each trachea of the more *ventral* set also divides into two branches, one of which becomes a secondary efferent of the latero-dorsal gill-fold, the other a secondary efferent of the latero-ventral gill-fold.

Each visceral trunk, on the other hand, gives off *only one* set of primary efferent tracheæ. Each of these tracheæ divides into two branches, one becoming a secondary efferent of the latero-ventral gill-fold, the other a secondary efferent of the ventral gill-fold.

In other words, the two dorsal trunks between them supply the whole tracheal system for the dorsal and two latero-dorsal gill-folds, and the upper half of that for each of the latero-ventral gill-folds. The two visceral tracheæ between them supply the whole tracheal system for the ventral gill-fold and the lower half of that for each of the latero-ventral gill-folds.

If, now, we admit the trachea *lvb* as portion of the visceral system (with which it becomes continuous in later stages), we may say that the dorsal trunks account for the *upper two-thirds* of the tracheal system of the rectum, the visceral trunks for the *lower one-third*. The *proportions* are made up as follows :—

Gill-fold.	Branches from Dorsal Trunks.	Branches from Visceral Trunks.
Dorsal	2	0
Right latero-dorsal ..	2	0
Left latero-dorsal	2	0
Right latero-ventral ..	1	1
Left latero-ventral ..	1	1
Ventral	0	2
Total	8	4

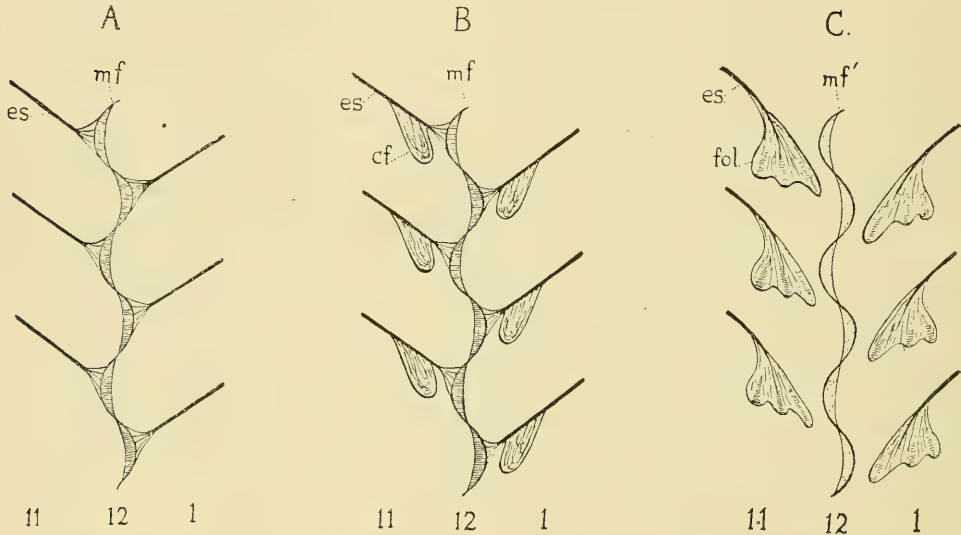
(3) *The gradual development of the duplex system of gills.*
(Text-fig. 20.)

Passing now to the third instar, we notice the following advances made in the gill-basket of a transparent larva examined soon after ecdysis :—

All the gill-folds show deeper undulations, and the tracheal loops are more abundant.

The efferent trachææ are considerably stouter.

There is thus very little advance on the stage seen in the second instar. When, however, we come to the fourth instar (text-fig. 20, B), we find a very interesting stage in the development. Confining our studies for the



TEXT-FIG. 20.—Diagrammatic representation of dorsal portion of gill-basket, viewed from above, to show the development of the Duplex System from a primitive Simplex System. Taken from camera-lucida drawings immediately after ecdysis. A. From *Anax papuensis*, Burm., 2nd Instar; primitive Simplex Main Fold. B. From *Anax papuensis*, Burm., 4th Instar; development of cross-folds. C. From *Æschna brevistyla*, Ramb., 7th Instar; abortion of tracheal supply to main fold, and growth of cross-folds to form foliate gills of the Duplex System (7th Instar of *Anax* the same, with papillæ). *cf*, cross-fold; *es*, secondary efferent trachea; *fol*, folia; *mf*, main fold; *mf'*, ditto, aborted; 11, 12, 1, positions on clock-face.

present to the dorsal gill-fold, we notice the following very interesting points :—

The undulations of the main gill-fold are deep and very regular. Each secondary efferent trachea gives off its branch trachææ to the fold along the crest of a wave, *i. e.* to that portion of the fold which is convex to it. From each secondary efferent trachea, close to the main fold, there rises up

(projecting into the rectal cavity in a slanting direction, somewhat posteriad) a small but very distinct oval leaflet filled with tiny tracheal loops. These leaflets are unmistakably similar both in appearance and position to the *lamellæ* of the Libellulidæ. They also correspond fairly closely to the *cross-folds* in the *Simplex System* of gill-folds.

With these leaflets in front of us, we stand at the parting of the ways between the Undulate Simplex and the Lamellate Duplex Types. If the leaflets, in their further development, joined up to the main fold as a series of supports, we should obtain the *Undulate Simplex* type of gill-fold seen in *Petalura*, *Cordulegaster*, and *Austrogomphus*. If, on the other hand, the main folds became aborted, and the leaflets developed into lamellæ of considerable size, we should then have the *Lamellate Duplex* type seen in the Libellulidæ.

In order to study the formation of the gill-basket at the fourth instar more fully, several larvæ of *Æschna* and *Anax* were taken immediately after ecdysis and prepared for sectioning. Text-fig. 1 shows a cross-section through a larva of *Æschna brevistyla* at about the middle of the gill-basket. Owing to the semi-collapsed state of the main folds, especially on the ventral side, the undulations are flattened down, and several are cut through in the same section. This accounts for the zigzag course of the sections across these main folds.

Between each pair of main folds two small triangular prominences (*cf*) are to be seen, appearing as slight evaginations of the rectal epithelium. Their size depends, of course, on whether the particular section studied cuts the leaflets through their middle or near one of their edges. The largest cross-section of a leaflet seen in this series of sections was somewhat larger than the two marked *cf* in the figure.

A careful examination of sections of a larva in the *third instar* shows that these leaflets really begin to develop at that stage as tiny outpushings of the rectal epithelium, between, and fairly close up to, the main folds. They are not, however, large enough to be noticed in an examination of the living larva. Sections of larvæ in the second instar, on the other hand, show no sign of these outpushings. The only other noticeable differences between sections taken from larvæ of the second, third, and fourth instars are the gradual increase in definiteness and depth of the undulations in the main folds and the rapid increase in size in the larger tracheæ.

From the fourth instar onwards the small leaflets rapidly increase in size, while numerous tracheoles are seen to ramify in them. On the other hand, the tracheæ entering the main folds become inconspicuous and are soon lost to view, in the live larva, in the mass of yellowish-brown epithelium which seems to be raised up along the course of the original main folds during the rapid growth of the leaflets. At the sixth instar these leaflets are very

clearly to be distinguished by their abundant tracheation and by the gradual appearance of a darkish pigmentation localizing their position.

Larvæ of both *Æschna* and *Anax* were taken and sectioned at the seventh instar (text-fig. 20, C). Also, the gill-baskets of others of the same age were opened, and selected portions of the gills photographed, when a very interesting result was noticeable. In both genera the foliate type of gill was completely established by the development of undulations on the original leaflets, which by now project into the rectum in the form of a series of definite foliæ closely resembling those seen in the full-grown larva of *Æschna*, but not so deeply pigmented. But, in *Anax*, little bundles of developing papillæ were to be seen projecting outwards from the free edges of the foliæ.

In order to trace back the origin of these papillæ, I took the only remaining larva of *Anax* which I still possessed in the *sixth* instar, and dissected it carefully. On opening the gill-basket I found, at the extreme edges of the foliæ, tiny groups of four or five papillæ grouped together in a very immature stage of development, with here and there a small swelling indicating the beginning of papilla-formation along other parts of the leaf-edge. The papillæ are, at the first, small rather pyramidal tubercles, containing no tracheæ. As they increase in length (their area in cross-section near the base remaining fairly constant), the tracheal loops lying immediately beneath them are as it were *sucked up* out of their course into the papillæ. Finally, they become long enough to resemble the finger of a glove, with the capillary loops rising up well into their interior. At the sixth instar I did not notice a single papilla fully formed, and very few indeed had tracheal loops in them. At the seventh instar a large number of the papillæ were fairly complete, others were in all stages of development.

The further development of the typical papillo-foliate gill-type in *Anax* is very rapid. During the seventh instar the foliæ tend to curve over posteriorly along their free edge, while numerous papillæ arise at all points of the curve. At the eighth instar this curved folia already bears a very close resemblance to the *basal hump* of the full-grown larva, and is very deeply pigmented. A series of these foliæ taken along the free edge of a hemibranch in semi-profile, at the eighth instar, is shown in Plate 19. fig. 8. As will be seen, they differ little from the condition seen in the adult (Plate 21. fig. 16).

PHYLOGENETIC CONCLUSIONS. (Text-fig. 21.)

We are now in a position to review our study of the gill-basket with a view to stating the phylogenetic conclusions to which it points. These may be summarized as follows :—

1. The most primitive type of gill-basket to be found in present-day Anisopterid larvæ is undoubtedly the *Undulate Type* in the *Simplex System*.

This is shown by two facts :—

(a) The *Undulate Type* is found in the mature larvæ of just those very genera which are universally acknowledged, by reason of the number of other archaic characters which they possess, to be the most primitive forms of Anisoptera still existing (*Petalura*, *Cordulegaster*, *Austrogomphus*).

(b) The *Undulate Type* also occurs in the young larvæ of *Æschna* and *Anax*, which later on develop a *Duplex System* of gills of the *Foliate Type*.

2. The main longitudinal gill-folds in the Simplex System are homologous with the so-called "rectal glands." This is also shown by two facts :—

(a) Both occur as six longitudinal eversions of the rectal wall in the positions 2, 4, 6, 8, 10, 12 on the clock-face.

(b) The aborted main folds in the well-grown larva of *Æschna* can be followed back into the "rectal glands," of which they form the anterior portion.

3. The cross-folds in the Simplex System arise, ontogenetically, later than the main folds. They are therefore probably less archaic in origin than the main folds.

4. The *Papillate Type* of gill in the Simplex System is a remarkable specialization from the more primitive *Undulate Type*. It may be regarded as the highest expression of the Simplex System.

5. The *Duplex System* arose from the more primitive *Simplex System* (early *Undulate Type*) by gradual suppression of the main folds and by the elevation of the function of the cross-folds to act as the principal gills of the system. This is shown by the two following facts :—

(a) In the ontogenetic development of the *Duplex System* in *Æschna* and *Anax* a gradual suppression of the main folds and upgrowth of the cross-folds leads to the development of the *Foliate Type* of gill from the *Undulate Type* possessed by the young larva.

(b) The separate gills of the hemibranchs of the *Duplex System* correspond exactly, in their position and tracheal supply, with the cross-folds in the *Simplex System*.

6. The fact that the middle line or axes of symmetry of the holobranchs in the *Duplex System* occupy the positions 1, 3, 5, 7, 9, 11 on the clock-face, cannot be used as an argument against the homology of the rectal gills with the "rectal glands," as Sadones has used it. The change of position is clearly explained by 5.

7. The *Implicate Type* of the *Duplex System* is the most archaic type still extant in that system. This is shown by :—

(a) Its resemblance in details to the *Undulate Type*.

(b) The very weak development of the basal pads.

8. The *Implicate Type* does not, however, lie exactly along the direct

phyletic line of ascent of the *Foliate Type*, rather the latter, as formed in *Æschna* and *Anax*, must be considered to have arisen from a somewhat simpler development of the *Implicate Type*.

9. The *Foliate Type* represents the highest development of the *Duplex System* within the *Æschninae*. The *papillo-foliate* sub-type is a specialization from the *normal foliate* sub-type of *Æschna*.

10. The *Lamellate Type* is an isolated type within the *Duplex System*, of very high specialization, but undoubtedly also of very great antiquity. Its point of phyletic union with the other gill-types must be sought for far back in the early days of the *Undulate Type*, before the main folds attained any great development. In the formation of the *Lamellate Type* the main folds became completely suppressed, the cross-folds became highly developed as gill-lamellæ, and the basal pads underwent a correlated localization as flat supporting discs and became deeply coloured.

11. The number of undulations and corresponding sets of cross-folds corresponded primarily with the number of primary efferent tracheæ developed, and was probably at the first from six to nine. This number early increased to twelve, the number still found in the mature larvæ of *Petalura*, *Cordulegaster*, *Austrogomphus*, *Hemigomphus*, *Anax*, and *Synthemis*. Further development increased this number up to as much as thirty in highly specialized cases (e. g., *Diplacodes*).

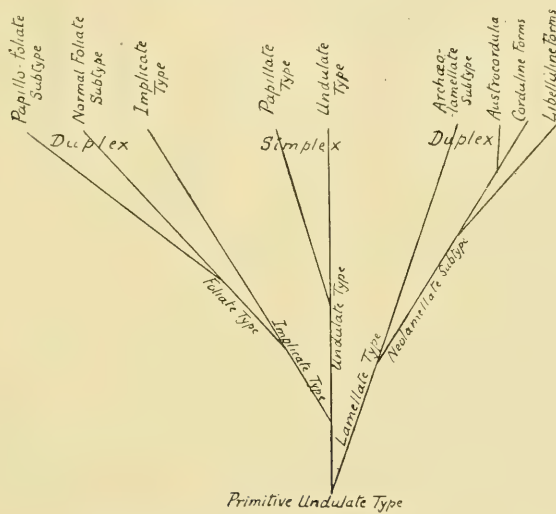
12. Within the *Lamellate Type* only minor variations of structure occur. We can perhaps distinguish as sub-types the *Archilamellate* gills in the *Synthemini* and the *Neolamellate* gills in the rest of the *Libellulidæ*. Within the latter sub-type some minor differences separate the *Corduliinae* from the *Libellulinae*. The latter subfamily shows the higher specialization, culminating in *Diplacodes*. *Austrocordulia* has struck out a side-line of specialization by an attempt to develop papillæ. *Cordulephyia* appears, as in other larval characteristics, to belong definitely to the *Eucorduliini*.

The above twelve conclusions are illustrated by the phylogenetic diagram in text-fig. 21.

The value of the evidence afforded by the gill-structure in elucidating the general phylogeny of the Anisoptera is considerable, and tends to strengthen the views already put forward by recent workers in that field. In pointing to *Petalura* and *Cordulegaster* as the most archaic of living Anisoptera, it agrees with the conclusions already obtained both from Palæontology and from other branches of the Comparative Morphology of both larva and embryo. The greater antiquity of *Austrogomphus* as compared with *Hemigomphus*, and hence also with *Gomphus* and *Onychogomphus*, is a very interesting point, though only of secondary importance. Passing on to the *Æschninae*, it is much to be regretted that a larva of the archaic tribe *Petaliini* cannot be obtained for study, since this will almost certainly either give us the missing link between the *Implicate Type* and the *Undulate Type*,

or provide us with another new type. In passing, it may be remarked that, if the larva of the *Petaliini* can be shown to possess gills of the *Duplex System*, that fact will practically decide once and for all the claim of this group—already a strong one—to be included in the true *Æschninae*. The study of the *Implicate Type* as compared with the *Foliate Type* confirms the view already held that the *Brachytronini* (*Implicate Type*) are more primitive than the *Æschnini*, though they have branched away somewhat from the line of ascent of the latter. The study of the *Foliate Types* marks out *Æschna* and *Anax* as naturally closely allied, and also confirms the view already held that *Anax* as now constituted was formed by rapid cænogenetic specialization from the older *Æschna*-stock.

The most valuable phylogenetic evidence afforded by our study is undoubtedly that which concerns the Libellulid stock, whose origin is still



TEXT-FIG. 21.—Phylogenetic Diagram.

a matter of doubt. The form of the gill-basket points at once to the two facts that they are not only a very highly specialized side-branch from the main line of Anisopterid advance (which we may take to be represented by the upgrowth of the *Æschninae* from Undulate-Typed ancestors), but that the origin of this highly successful stock goes very far back, almost to the very root-beginnings of Anisopterid history. By no other supposition than this can we explain the complete suppression of the underlying *Undulate Type* even as early as the third or fourth instar in the Libellulid larva. Further light may be thrown on this by studying the gill-basket in the newly-hatched Libellulid larva; we may even have to examine its formation within the embryo. Even within the evidence before us, we are able to single out

Cordulegaster (on its totality of larval characteristics) as the nearest living relative of this huge family. But the gap between *Cordulegaster* and the Libellulidæ is still a large one. This gap is considerably lessened if we compare *Cordulegaster* with *Synthemis*, for the gill-basket of the latter agrees with that of the former in possessing only the archaic number of twelve sets of cross-folds, albeit they are wholly disguised as lamellæ. The large size also of the basal pads indicates to us a way of approach between the localized pads of Libellulidæ and the generalized pads of other Anisoptera. It seems probable, perhaps, that we shall in the end have to admit that the origin of the Libellulidæ is quite lost to us in the mists of antiquity, unless some fortunate fossil find in the still unworked fresh-water beds of the Trias-Jura in Australia may yet hold the key to the puzzle.

Finally, our study emphasizes the homogeneity and closeness of descent of the two subfamilies forming the Libellulidæ. The characters separating the *Corduliinæ* from the *Libellulinaæ* are everywhere of a lower order of value than those separating other subfamilies. Yet there can be no doubt of their distinctness. It seems, too, that much of the difficulty of separating the two may be due to convergence between their highest members. Certainly the difference between *Synthemis* and, say, *Libellula* is very much more marked than that between the *Eucorduliini* and *Trameïni*, which are regarded at present as the highest members of the two subfamilies. We may, perhaps, have to alter our views later on as to the position of the *Trameïni*, and assign to the *Sympetrini*—those Passerines amongst Odonata—the pride of place in the Libelluline stock.

In conclusion, if we had to select from amongst all the forms studied the two most highly specialized larvæ, we should undoubtedly choose *Anax* and *Diplacodes*, standing at the extreme ends of development of their respective branches. To select from these two the most highly specialized of all Anisopterid larvæ would be no easy task; but I am inclined to award the palm to *Anax*, which seems to me to combine in the highest degree all those qualities essential to the carnivorous aquatic larva.

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EXPLANATION OF THE PLATES.

PLATE 18.

(Simplex and Implicate Types. Photomicrographs.)

- Fig. 1. *Austrogomphus ochraceus*, Selys. Undulate Simplex Gill, from full-grown larva.
2. *Hemigomphus heteroclitus*, Selys. Papillate Simplex Gill, from nearly full-grown larva.
3. *Austroæschna multipunctata*, Martin. Implicate Duplex Gill, from full-grown larva.
4. *Dendroæschna conspersa*, Tillyard. Ditto.
- (All figures $\times 60$.)

PLATE 19.

(Foliate Types. Photomicrographs.)

- Fig. 5. *Æschna brevistyla*, Ramb. Normal Foliate Gills, from anterior portion of gill-basket of a larva about the 10th instar.
6. *Æschna brevistyla*, Ramb. Ditto, from posterior portion of gill-basket of same larva.
7. *Anax papuensis*, Burm. Papillo-foliate Gills, from larva at 8th instar.
8. *Anax papuensis*, Burm. The same, seen in profile, from larva at 7th instar.
- (All figures $\times 60$.)

PLATE 20.

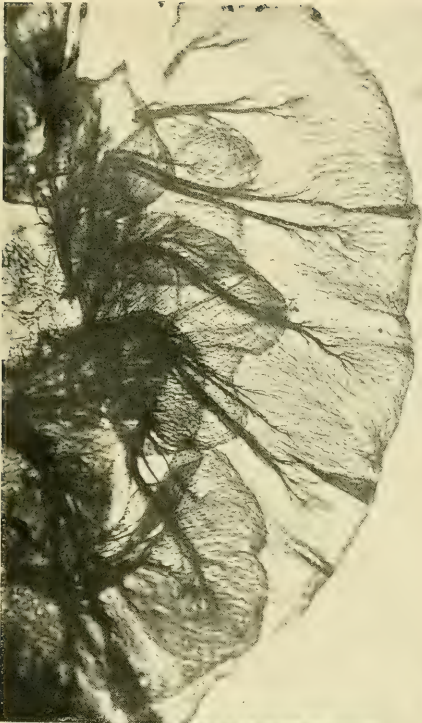
(Lamellate Types. Photomicrographs.)

- Fig. 9. *Metathemis guttata*, Selys. Lamellate Gills, from full-grown larva.
10. *Synthemis macrostigma*, Selys. Ditto.
11. *Austrocordulia refracta*, Tillyard. Ditto.
12. *Hemicordulia tau*, Selys. Ditto.
- (Fig. 10, $\times 75$. Figs. 9, 11, 12, $\times 60$.)

PLATE 21.

(Miscellaneous Photomicrographs.)

- Fig. 13. *Orthetrum caledonicum*, Br. Lamellate Gills, from full-grown larva. ($\times 60$.)
14. *Orthetrum caledonicum*, Br. Ditto. ($\times 16$.)
15. *Æschna brevistyla*, Ramb. Normal Foliate Gills, from full-grown larva (posterior portion of gill-basket). ($\times 16$.)
16. *Anax papuensis*, Burm. Papillo-foliate Gills, from full-grown larva. In profile. ($\times 16$.)
17. *Austrogomphus ochraceus*, Selys. Portions of two holobranchs, to show cross-folds. ($\times 16$.) Compare text-fig. 2.



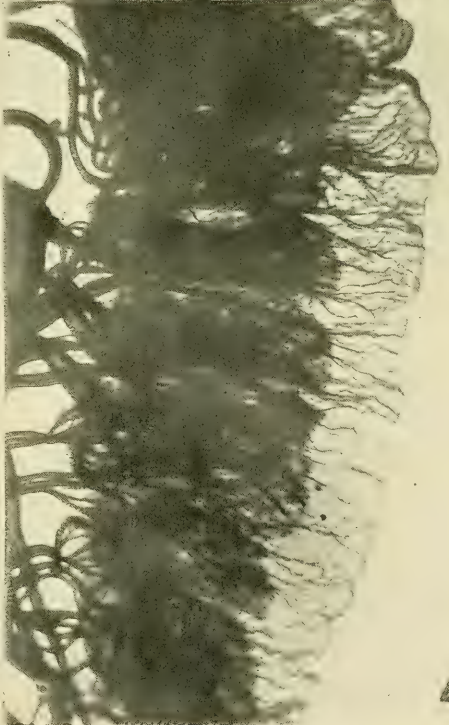
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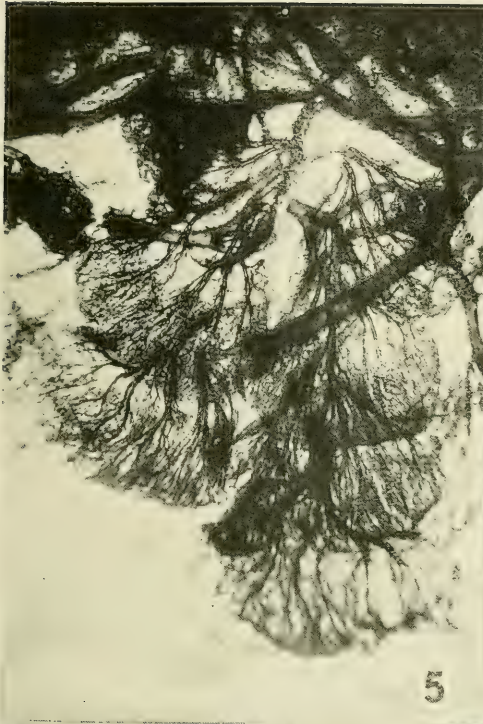


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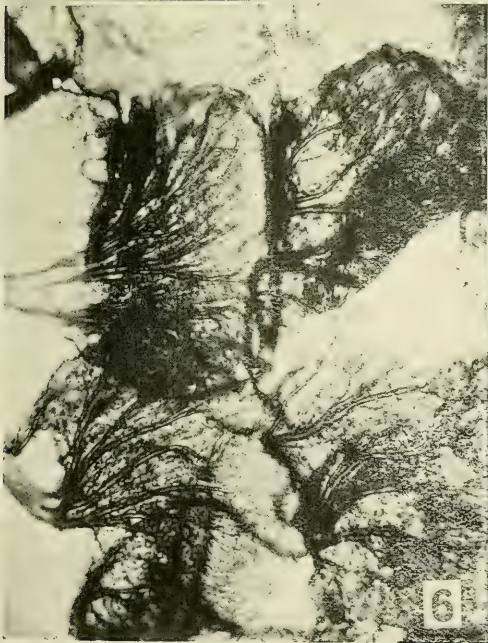
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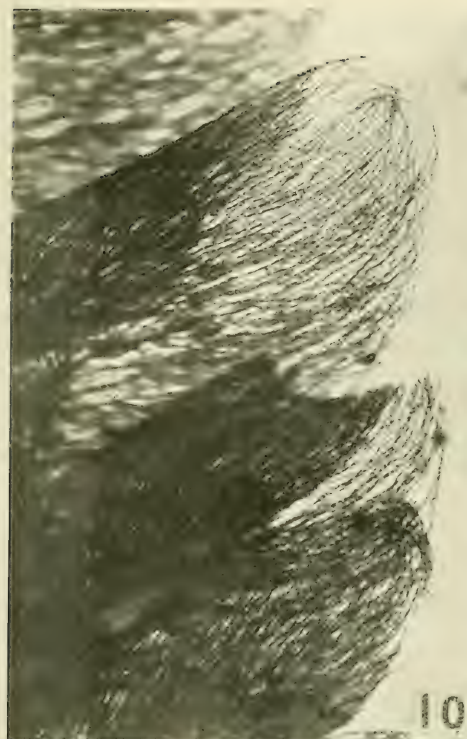


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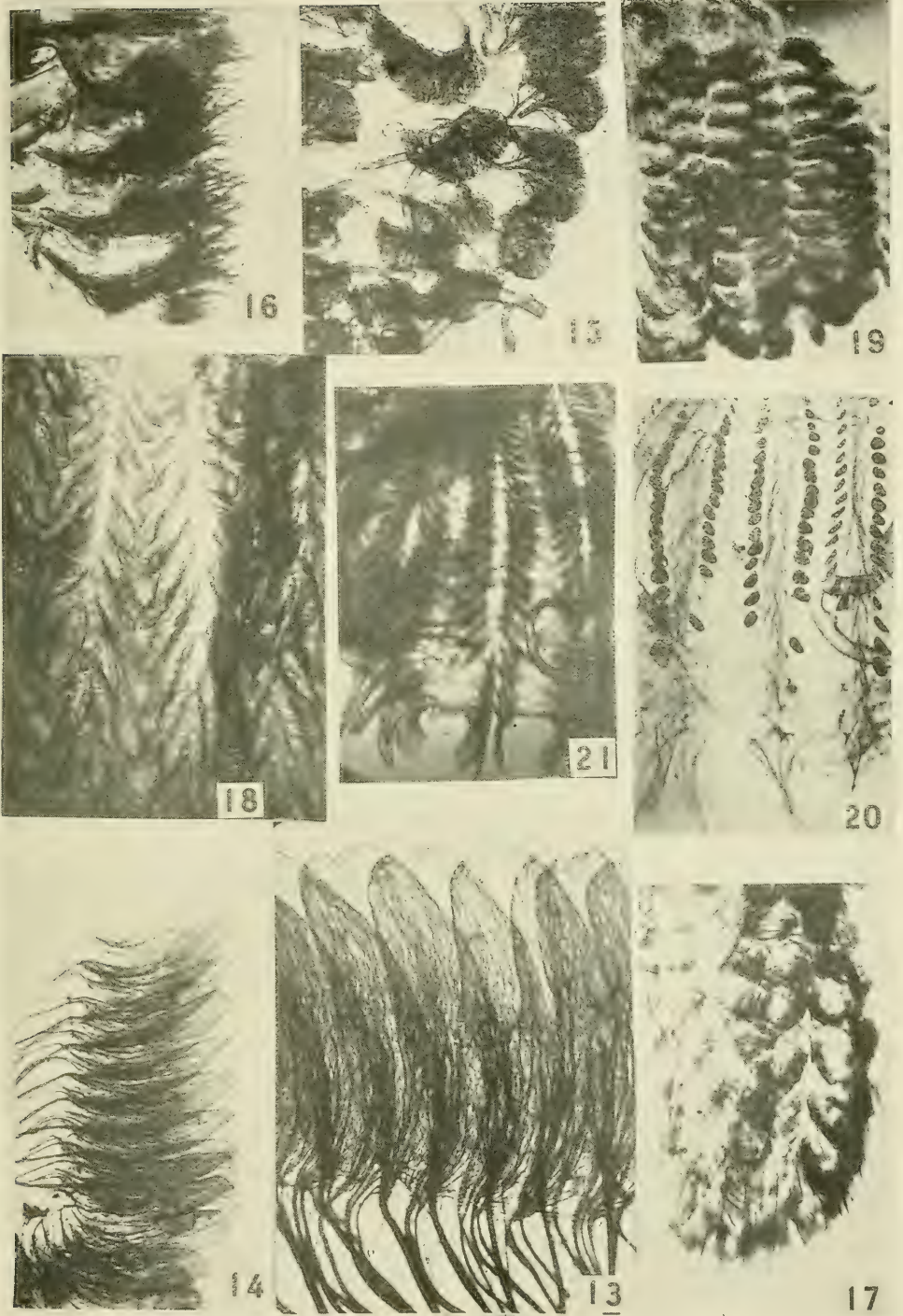


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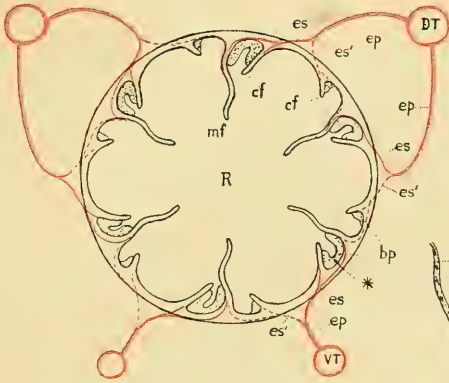
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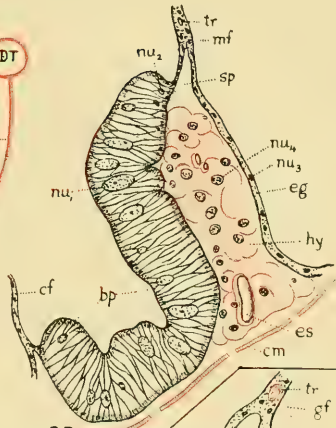
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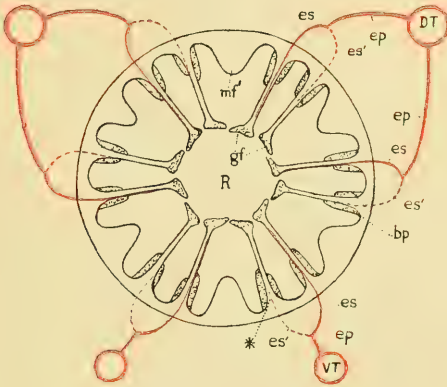
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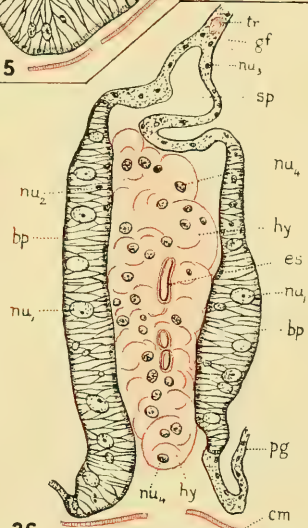
22 *Austrogomphus ochraceus*



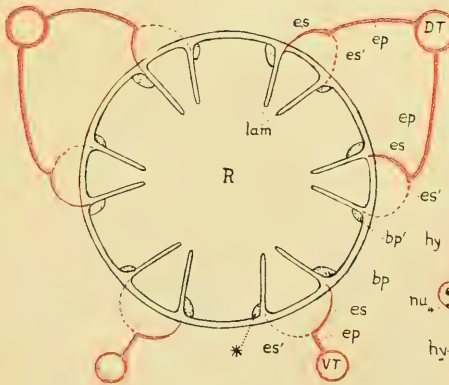
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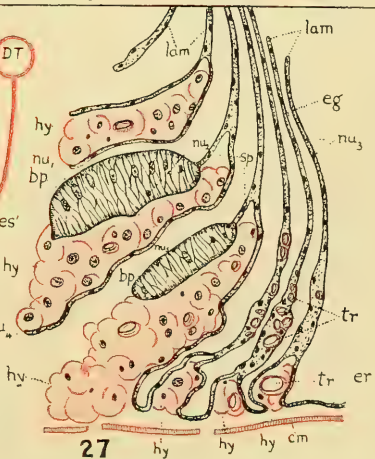
23 *Aeschna brevistyla*



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24 *Cordulephya pygmaea*



27

Grout, Sc.

RECTAL BREATHING APPARATUS.

- Fig. 18. *Austrogomphus multipunctata*, Martin. Portions of three holobranchs. ($\times 16$) Compare text-fig. 5.
19. *Anax papuensis*, Burm. Three complete holobranchs. ($\times 16$) Compare text-fig. 9.
20. *Austrocordulia refracta*, Tillyard. Portions of three holobranchs. ($\times 16$) Notice basal pads and absence of pigmentation.
21. *Diplacodes hæmatodes*, Burm. Portions of three holobranchs. ($\times 16$) Notice basal pads and deep pigmentation. Compare text-fig. 12.
- (Figs. 17, 18, 19, 21 are photographs of portions of the whole mounts from which the text-figures 2, 5, 9, 12 respectively were also taken.)

PLATE 22.

(Transverse sections of Gill-baskets and Basal Pads.)

- Fig. 22. *Austrogomphus ochraceus*, Selys. Diagrammatic. T. S. through gill-basket. ($\times 46$)
23. *Æschna brevistyla*, Ramb. Ditto. ($\times 16$)
24. *Cordulephya pygmaea*, Selys. Ditto. ($\times 22$) Only those lamellæ are shown whose basal pads are actually cut, in this section, nearest their middle lines. The rest are omitted. Contrast fig. 27.
25. *Austrogomphus ochraceus*, Selys. Much enlarged T. S. through base of gill marked * in fig. 22. ($\times 305$.)
26. *Æschna brevistyla*, Ramb. Much enlarged T. S. through base of gill marked * in fig. 23. ($\times 135$.)
27. *Cordulephya pygmaea*, Selys. Much enlarged T. S. through base of lamellæ actually cut through near point marked * in fig. 24. ($\times 220$.)

Reference letters:—

bp, basal pad; *bp'* (fig. 24), the same, cut farther from its middle line; *cf*, cross-fold; *cm*, circular muscle-layer; *DT*, dorsal tracheal trunk; *eg*, epithelium of gill; *ep*, primary efferent trachea; *er*, epithelium of rectum; *es*, secondary efferent trachea; *es'*, the same (dotted line), not in the true level of the section; *gf*, gill-folia; *hy*, hypobranchial tissue; *lam*, gill-lamella; *mf*, main longitudinal gill-fold; *mf'* (fig. 23), the same, aborted and functionless; *nu₁*, meganucleus of basal pad; *nu₂*, micronucleus of same; *nu₃*, nucleus of gill-epithelium; *nu₄*, nucleus of hypobranchial tissue; *nu₅* (fig. 27), minute nucleus in basal pad; *pg* (fig. 26), highly pigmented gill-epithelium; *R*, rectal cavity; *sp*, space between gill-walls, above hypobranchial tissue; *tr*, trachea; *VT*, visceral tracheal trunk.

POSTSCRIPT, March 1st, 1915.

Ontogeny of the Gills in the Larvæ of Libellulidæ.

Since the above paper was written, I have succeeded in hatching a number of larvæ of *Diplacodes hæmatodes*, Burm., and have studied their gill-formation. Two females of this species were taken while ovipositing in the George's River, Ingleburn, N.S.W., on Feb. 13th last. Each laid a large number of eggs into a tube of water held in the hand. These eggs were placed with sand and débris in water in a Petri dish. In a few hours they became coagulated together into large masses. Within three days they developed a strong growth of fungus, so that I did not expect them to hatch.

However, on examining them nine days after they were laid, on Feb. 22nd, I found about two hundred newly-hatched larvæ in the dish. Some of these were quite transparent and freshly hatched. The gill-basket is very peculiar. It is of the *Simplex Undulate Type*, the main folds being clearly developed. Each main fold is brownish in colour and *very short*. On either side of it, *only one or two cross-folds* are developed. These are well supplied with tracheal capillaries, and are already recognizable as lamellæ by their size, shape, and inclination to the body-axis. No ecdyses having so far occurred, I cannot say definitely how the increase in the number of lamellæ takes place. From the position of the gill-basket, it seems likely that the first-formed lamellæ are the hindmost, and that others will be developed from behind forwards.

The above observation justifies the phylogenetic conclusions at which I had already arrived, that the *Simplex Undulate Type* is the most primitive type of Anisopterid rectal gill. At the same time, it marks off the *Lamellate Type* as very distinct in its origin, and apparently developed at an earlier period in the history of Odonata than were the other *Duplex Types* found in the *Æschinæ*.—R. J. T.
